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Management of the western flower thrips on strawberry

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Management of the western flower thrips on strawberry

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Abstract

The western flower thrips, *Frankliniella occidentalis* (Pergande), is an increasing problem in UK strawberry crops. The use of polythene tunnels has provided a more favourable environment for the pest, and pesticide-resistant strains have resulted in control failure. There is a need for improved knowledge of thrips biology and for additional control methods that can be integrated with natural enemies in order to make thrips management programmes more robust. The distribution of, and damage caused by, *F. occidentalis* was investigated to improve monitoring and decision-making, and the viability of using traps as a control was tested. Over 74% of adult thrips on plants were in flowers. Twice as many adult thrips were found in mature flowers at the top of the plant compared to those at the side. The distribution of larvae between flower and fruit stages varied with thrips density. All stages of flower and fruit were susceptible to damage but thrips larvae caused more damage than adults per individual, so the distribution and numbers of larvae between fruit stages best predicted the timing of damage. The predatory mite *Neoseiulus cucumeris* Oudemans reduced damage by feeding on thrips larvae. Economic crop loss occurred at five adult thrips per flower in the absence of *N. cucumeris*, but up to about 11 adult thrips per flower with good mite establishment. Adult *F. occidentalis* females overwintered on strawberry and on weeds, resulting in more thrips in second-year than in first-year crops. Mass trapping using blue sticky roller traps caught sufficient adult thrips to reduce fruit damage by 55-68% and increased grower returns by an estimated £2.2k per hectare. The addition of the *F. occidentalis* aggregation pheromone, neryl (*S*)-2-methylbutanoate, to the traps doubled the trap catch, but a visual stimulus was essential for trapping. (*R*)-lavandulyl acetate reduced trap catch, suggesting that it is not part of the aggregation pheromone.

Table of Contents

| | |
|---|-----------|
| Abstract | i |
| Table of contents | ii |
| Tables and Figures | viii |
| List of species cited..... | ix |
| List of abbreviations..... | xi |
| Acknowledgements | xii |
| Papers published | xiii |
| | |
| Chapter 1 General introduction | 1 |
| 1.1. The biology of <i>Frankliniella occidentalis</i> | 1 |
| 1.2. Pest status of <i>Frankliniella occidentalis</i> | 6 |
| 1.3. Management of <i>Frankliniella occidentalis</i> | 8 |
| 1.4. Aims of the study | 13 |
| | |
| Chapter 2 General methods | 18 |
| 2.1. Introduction | 18 |
| 2.2. Site details and growing systems | 18 |
| 2.3. Thrips identification | 19 |
| 2.4. Counting and removing thrips from traps | 22 |
| 2.5. Validation of the method used for sampling strawberry flowers..... | 22 |
| 2.5.1. Flower sampling: materials and methods | 23 |
| 2.5.1.1. Which flower should be sampled?..... | 23 |
| 2.5.1.2. Is the sampling method accurate and consistent between samplers? | 24 |
| 2.5.2. Flower sampling: results | 24 |
| 2.5.2.1. Which flower should be sampled?..... | 24 |
| 2.5.2.2. Is the sampling method accurate and consistent between samplers? | 25 |
| 2.5.3. Flower sampling: discussion | 25 |
| 2.6. Environmental measurements | 28 |
| 2.7. Rearing of <i>Frankliniella occidentalis</i> | 28 |
| 2.8. Statistical analysis | 30 |

| | |
|---|---------------|
| Chapter 3 Phenology in strawberry | 39 |
| 3.1. Introduction | 39 |
| 3.2. Materials and Methods | 43 |
| 3.2.1. Seasonal abundance | 44 |
| 3.2.2. Which thrips species are present? | 46 |
| 3.2.3. Are there weed hosts within strawberry fields? | 47 |
| 3.2.4. Do active stages of <i>F. occidentalis</i> overwinter? | 48 |
| 3.2.5. Between-field distribution and abundance: is there a difference in thrips distribution and abundance between first and second-year crops? | 50 |
| 3.2.6. Within-field distribution and abundance: do temperature gradients within fields affect local abundance? | 50 |
| 3.2.7. Movement of adults between flowers | 52 |
| 3.2.8. Statistical analysis | 53 |
| 3.3. Results | 53 |
| 3.3.1. Seasonal abundance | 53 |
| 3.3.2. Which thrips species are present? | 55 |
| 3.3.3. Are there weed hosts within strawberry fields? | 55 |
| 3.3.4. Do active stages of <i>F. occidentalis</i> overwinter? | 56 |
| 3.3.5. Between-field distribution and abundance: is there a difference in thrips distribution and abundance between first and second-year crops? | 57 |
| 3.3.6. Within-field distribution and abundance: do temperature gradients within fields affect local abundance? | 58 |
| 3.3.7. Movement of adults between flowers | 59 |
| 3.4. Discussion | 59 |
| Chapter 4 Damage to strawberry fruit | 83 |
| 4.1. Introduction | 83 |
| 4.2. Materials and Methods | 87 |
| 4.2.1. What is the effect of <i>F. occidentalis</i> on strawberry fruit damage? | 88 |
| 4.2.2. What is the effect of <i>N. cucumeris</i> on <i>F. occidentalis</i> fruit damage? | 89 |
| 4.2.2.1. At four adult thrips per flower | 89 |
| 4.2.2.2. At eight adult thrips per flower | 90 |
| 4.2.2.3. Adjustment of the Economic Injury Level (EIL) in the presence of <i>N. cucumeris</i> . | 90 |

| | | |
|----------|--|-----|
| 4.2.3. | When does fruit damage occur during fruit development? | 91 |
| 4.2.3.1. | Which flower or fruit stages are susceptible to adult or larval thrips damage? | 91 |
| 4.2.3.2. | Within-plant distribution of thrips | 92 |
| 4.2.3.3. | Flower progression in relation to <i>F. occidentalis</i> life cycle | 92 |
| 4.2.4. | Estimation of the EIL in a commercial pack-house | 92 |
| 4.2.5. | Validation of EILs in commercial crops | 93 |
| 4.2.5.1. | What is the relationship between flowering periods, thrips per flower and fruit damage through the season? | 94 |
| 4.2.5.2. | EILs observed in commercial crops. | 95 |
| 4.2.6. | How many flowers should be sampled to estimate thrips density?..... | 95 |
| 4.2.7. | An action plan for growers | 96 |
| 4.2.8. | Statistical analysis | 96 |
| 4.3. | Results | 97 |
| 4.3.1. | What is the effect of <i>F. occidentalis</i> on strawberry fruit damage? | 97 |
| 4.3.2. | What is the effect of <i>N. cucumeris</i> on <i>F. occidentalis</i> fruit damage? | 97 |
| 4.3.2.1. | At four adult thrips per flower | 97 |
| 4.3.2.2. | At eight adult thrips per flower..... | 98 |
| 4.3.2.3. | Adjustment of the EIL in the presence of <i>N. cucumeris</i> | 98 |
| 4.3.3. | When does damage occur during fruit development? | 99 |
| 4.3.3.1. | Which flower or fruit stages are susceptible to adult or larval thrips damage? | 99 |
| 4.3.3.2. | Within-plant distribution of thrips | 99 |
| 4.3.3.3. | Flower progression in relation to <i>F. occidentalis</i> life cycle | 100 |
| 4.3.4. | Estimation of the EIL in a commercial pack-house | 101 |
| 4.3.5. | Validation of EILs in commercial crops | 101 |
| 4.3.5.1. | What is the relationship between flowering periods, thrips per flower and fruit damage through the season? | 101 |
| 4.3.5.2. | EILs observed in commercial crops. | 103 |
| 4.3.6. | How many flowers should be sampled to estimate thrips density?..... | 103 |
| 4.3.7. | An action plan for growers | 104 |
| 4.4. | Discussion | 105 |

| | |
|--|------------|
| Chapter 5 Optimising pheromone use for trapping | 131 |
| 5.1. Introduction | 131 |
| 5.2. Materials and Methods | 138 |
| 5.2.1. Does the release rate of neryl (<i>S</i>)-2-methylbutanoate affect trap catch? | 142 |
| 5.2.2. Does the chiral form of neryl -2-methylbutanoate affect trap catch? | 143 |
| 5.2.3. Does lavandulyl acetate increase trap catch? | 144 |
| 5.2.3.1. Does the ratio of neryl (<i>S</i>)-2-methylbutanoate: (<i>R</i>)-lavandulyl acetate affect trap catch? | 144 |
| 5.2.3.2. Does the release rate of neryl (<i>S</i>)-2-methylbutanoate: (<i>R</i>)-lavandulyl acetate (5:1) affect trap catch? | 145 |
| 5.2.3.3. Does the chiral form of lavandulyl acetate affect trap catch? | 145 |
| 5.2.4. Is the pheromone trap catch enhanced by certain trap colours? | 146 |
| 5.2.5. Is the pheromone trap catch enhanced by trap type? | 146 |
| 5.2.6. Does trap height above the crop affect pheromone trap catch? | 147 |
| 5.2.7. Does trap spacing affect pheromone trap catch? | 147 |
| 5.2.8. Is response to pheromone synergised by plant volatiles? | 148 |
| 5.2.8.1. In a pepper crop in Spain | 148 |
| 5.2.8.2. In a strawberry crop in the UK | 149 |
| 5.2.9. Statistical analysis | 150 |
| 5.3. Results | 151 |
| 5.3.1. Does the release rate of neryl (<i>S</i>)-2-methylbutanoate affect trap catch? | 151 |
| 5.3.2. Does the chiral form of neryl -2-methylbutanoate affect trap catch? | 151 |
| 5.3.3. Does lavandulyl acetate affect trap catch? | 152 |
| 5.3.3.1. Does the ratio of neryl (<i>S</i>)-2-methylbutanoate: (<i>R</i>)-lavandulyl acetate affect trap catch? | 152 |
| 5.3.3.2. Does the release rate of neryl (<i>S</i>)-2-methylbutanoate: (<i>R</i>)-lavandulyl acetate (5:1) affect trap catch? | 152 |
| 5.3.3.3. Does the chiral form of lavandulyl acetate affect trap catch? | 153 |
| 5.3.4. Is the pheromone trap catch enhanced by certain trap colours? | 153 |
| 5.3.5. Is the pheromone trap catch enhanced by trap type? | 154 |
| 5.3.6. Does trap height above the crop affect pheromone trap catch? | 154 |
| 5.3.7. Does trap spacing affect pheromone trap catch? | 155 |
| 5.3.8. Is response to pheromone synergised by a plant volatile analogue? | 155 |

| | |
|---|-----|
| 5.3.8.1. In a pepper crop in Spain | 155 |
| 5.3.8.2. In a strawberry crop in the UK | 156 |
| 5.3.9. Thrips species composition | 156 |
| 5.4. Discussion | 157 |

Chapter 6 Can mass trapping reduce thrips damage and is it economically viable? 181

| | |
|---|-----|
| 6.1. Introduction | 181 |
| 6.2. Materials and Methods | 186 |
| 6.2.1. What is the effect of trap colour on trap catch of pest and beneficial insects? .. | 189 |
| 6.2.2. Can thrips escape from sticky traps? | 190 |
| 6.2.3. Do traps decrease in efficiency through time? | 191 |
| 6.2.4. Trap placement in commercial strawberry | 192 |
| 6.2.4.1. Does trap height and orientation affect trap catch? | 192 |
| 6.2.4.2. Are traps effective when placed between the tunnels? | 193 |
| 6.2.5. Are traps effective throughout the growing season? | 194 |
| 6.2.6. Can mass trapping reduce thrips numbers and fruit damage? | 195 |
| 6.2.6.1. Pilot experiments in a first-year and a second-year crop | 195 |
| 6.2.6.2. Mass trapping with roller traps, with and without the aggregation pheromone. | 198 |
| 6.2.6.3. Thrips identification | 200 |
| 6.2.7. Cost-benefit analysis of mass trapping with and without pheromone | 200 |
| 6.2.8. Statistical analysis | 201 |
| 6.3. Results | 202 |
| 6.3.1. What is the effect of trap colour on catch of pest and beneficial species? | 202 |
| 6.3.2. Can thrips escape from sticky traps? | 203 |
| 6.3.3. Do traps decrease in efficacy through time? | 203 |
| 6.3.4. Trap placement in commercial strawberry | 203 |
| 6.3.4.1. Does trap height and orientation affect trap catch? | 203 |
| 6.3.4.2. Are traps effective when placed between the tunnels? | 204 |
| 6.3.5. Efficiency of trapping through the season | 205 |
| 6.3.6. Can mass trapping reduce thrips numbers and fruit damage? | 205 |
| 6.3.6.1. Pilot experiment in a first-year and a second-year crop | 205 |
| 6.3.6.2. Mass trapping using roller traps with or without aggregation pheromone .. | 207 |

| | |
|---|------------|
| 6.3.7. Cost-benefit analysis of mass trapping with and without pheromone..... | 208 |
| 6.3.8. Thrips identification | 209 |
| 6.4. Discussion | 209 |
| Chapter 7 General discussion | 230 |
| 7.1. Monitoring thrips | 230 |
| 7.2. Economic Injury Levels | 231 |
| 7.3. Phenology in strawberry | 234 |
| 7.4. Optimising the use of the aggregation pheromone | 236 |
| 7.5. Mass trapping | 237 |
| 7.6. IPM of <i>F. occidentalis</i> in semi-protected strawberry..... | 238 |
| 8. References | 240 |
| Appendix A: Handout given to farm staff before sampling thrips in flowers | 277 |
| Appendix B: Temperatures recorded during the growing season, in a semi-protected strawberry crop in the West Midlands..... | 278 |

Tables and Figures

Because of the large numbers of tables and figures in this thesis, they are not indexed individually. Instead they are grouped at the back of each chapter so that they can be located easily as follows:

| Tables | Pages |
|---------------------|--|
| Tables 1.1 to 1.3 | Chapter 1, introduction.....15-17 |
| Tables 2.1 to 2.3 | Chapter 2, general methods.....31-33 |
| Tables 3.1 to 3.6 | Chapter 3, phenology in strawberry.....67-72 |
| Tables 4.1 to 4.5 | Chapter 4, damage to strawberry fruit.....114-118 |
| Table 5.1 | Chapter 5, optimising pheromone use for trapping.....165 |
| Tables 6.1 to 6.3 | Chapter 6, mass trapping.....216-218 |
| | |
| Figures | |
| Figures 2.1 to 2.5 | Chapter 2, general methods.....34-38 |
| Figures 3.1 to 3.10 | Chapter 3, phenology in strawberry.....73-82 |
| Figures 4.1 to 4.12 | Chapter 4, damage to strawberry fruit.....119-130 |
| Figures 5.1 to 5.15 | Chapter 5, optimising pheromone use for trapping.....166-180 |
| Figures 6.1 to 6.11 | Chapter 6, mass trapping.....219-229 |
| Figure 7.1 | Chapter 7, general discussion.....239 |

List of species cited

Thysanoptera

Aeolothrips intermedius Bagnall
Aeolothrips tenuicornis Bagnall
Echinothrips americanus Morgan
Frankliniella bispinosa (Morgan)
Frankliniella intonsa (Trybom)
Frankliniella occidentalis (Pergande)
Frankliniella tenuicornis (Uzel)
Frankliniella tritici (Fitch)
Kakothrips pisivorus (Westwood)
Oncothrips tepperi Karny
Pezothrips dianthi (Priesner)
Thrips angusticeps Uzel
Thrips atratus Haliday
Thrips fuscipennis Haliday
Thrips hawaiiensis (Morgan)
Thrips imaginis Bagnall
Thrips major Uzel
Thrips obscuratus (Crawford)
Thrips palmi Karny
Thrips tabaci Lindeman

Fungi

Beauveria bassiana (Balsamo) Vuillemin
Botrytis cinerea Kunze
Entomophthora thripidum Samson
Erysiphe cichoracearum
Metarhizium anisopliae Metsch.
Neozygites parvispora MacLeod & Carl

Plants

Brassica rapa Ssp oleifera de Candolle
Calystegia sepium Linnaeus
Capsella bursa-pastoris Linnaeus
Cerastium glomeratum Thuill.
Cucumis sativus Linnaeus
Dendranthema grandiflora Tzvelev
Fragaria X ananassa Duchesne
Galium aparine Linnaeus
Heracleum sphondylium Linnaeus
Impatiens walleriana Hook
Matricaria discoidea D. C.
Phaseolus vulgaris Linnaeus
Poa annua Linnaeus
Senecio vulgaris Linnaeus
Sisymbrium officinale (Linnaeus) Scop.
Solanum nigrum Linnaeus
Sonchus asper (Linnaeus) Hill
Stellaria media (Linnaeus) Vill.
Tagetes erecta Linnaeus
Taraxacum officinale Agg. Wigg.
Trifolium repens Linnaeus
Tripleurospermum inodorum Linnaeus
Urtica dioica Linnaeus
Verbena officinalis Linnaeus
Veronica persica Poiret
Vicia faba Linnaeus

Other invertebrate species

Agrilus planipennis Fairmaire

Agrotis segetum (Denis & Schiffermüller)

Amblyseius andersoni (Chant)

Anomala osakana Sawada

Anthocoris nemorum (Linnaeus)

Apis mellifera Linnaeus

Bombus terrestris (Linnaeus)

Carpophilus antiquus Melsheimer

Carpophilus mutilatus Erichson

Chrysoperla carnea (Stephens)

Conogethes punctiferalis Guenée

Cydia molesta (Busck)

Dalotia coriaria (Kraatz)

Dendroctonus ponderosae Hopkins

Drosophila suzukii (Matsumura)

Drosophila virilis Sturtevant

Geolaelaps aculeifer Canestrini

Gnathotricus salcatus (LeConte)

Heterorhabditis megidis (Poinar)

Ips pini (Say)

Lygus rugulipennis Poppius

Macrocheles robustulus (Berlese)

Musca domestica Linnaeus

Nauphoeta cinerea (Olivier)

Neoseiulus californicus (McGregor)

Neoseiulus cucumeris (Oudemans)

Orius insidiosus (Say)

Orius laevigatus (Fieber)

Orius majusculus (Reuter)

Phthorimaea operculella (Zeller)

Phytonemus pallidus (Banks)

Phytoseiulus persimilis Athias-Henriot

Planococcus ficus (Signoret)

Popillia japonica Newman

Prostephanus truncates (Horn)

Rhynchophorus palmarum (Linnaeus)

Steinernema feltiae (Filipjev)

Steinernema carpocapsae (Weiser)

Stratiolaelaps scimitus (Womersley)

Tetranychus urticae Koch

Thripinema nicklewoodi (Siddiqi)

Tribolium castaneum (Herbst)

Trichoplusia ni (Hübner)

List of abbreviations

| | |
|----------|--|
| ADAS | Agricultural Development and Advisory Service |
| ANOVA | ANalysis Of VAriance |
| AT | Action Threshold |
| CV | Coefficient of Variation |
| cv. | cultivar |
| DT | Damage Threshold |
| ee | enantiomeric excess |
| EIL | Economic Injury Level |
| GC | Gas Chromatography |
| GC-MS | Gas Chromatography - Mass Spectrometry |
| GLM | General Linear Model |
| GPS | Global Positioning System |
| INSV | Impatiens Necrotic Spot Virus |
| IPM | Integrated Pest Management |
| NRI | Natural Resources Institute |
| PEPI | Programs for EPIdemiologists |
| RAPD-PCR | Random Amplified Polymorphic DNA - Polymerase Chain Reaction |
| TSWV | Tomato spotted wilt virus |

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Papers published

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Sampson, C. & Kirk, W.D.J. (2012) Flower stage and position affect population estimates of the western flower thrips, *Frankliniella occidentalis* (Pergande), in strawberry. *Acta Phytopathologica et Entomologica Hungarica* 47, 133-139.

Sampson, C. & Kirk, W.D.J. (2013) Can mass trapping reduce thrips damage and is it economically viable? Management of the western flower thrips in strawberry. *PLOS ONE* 8, e80787.

Chapter 1

General introduction

1.1. The biology of *Frankliniella occidentalis*

Frankliniella occidentalis (Pergande) (Thripidae), the western flower thrips, belongs to the Thysanopteran insect order, commonly known as thrips. Fossil records of thrips date back to the Jurassic period and the approximately 5,500 species described today have diversified into fungal feeders, herbivores, predators and omnivores (Mound, 2005). Thrips are most abundant in the tropics, but are found throughout the world from Alaska (60°N) to New Zealand (45°S) (Lewis, 1973). They are separated from other insect orders by having a single mandible adapted for piercing and sucking (Heming, 1989) and an inflatable bladder (arolium) on their tarsi with which they hold onto surfaces (Heming, 1971). Thrips are characterised by the delicate fringed wings from which their name derives (thysanos = fringe, pteron = wing). Most are small and elongate, <2 mm long and display thigmotactic behaviour, congregating in small spaces when at rest, making them hard to contact with insecticides and difficult to detect for quarantine and monitoring purposes (Hansen *et al.*, 2003b). Despite or perhaps partly because of their small size, about a hundred species, mainly in the family Thripidae, are important crop pests (Lewis, 1997a). The most damaging species (about 10 in number) are vectors of tospoviruses as well as causing direct feeding damage (Mound, 2002), of which *F. occidentalis* is currently considered the most economically damaging on a global scale (Mantel & Vierbergen, 1996).

Native to the western USA (Bailey, 1938), *F. occidentalis* has spread rapidly around the world since the 1970s, probably as a result of pesticide-resistant biotypes being transported through the plant-trade in an increasingly global market (Kirk & Terry, 2003). Recent work has revealed that there are two species of *F. occidentalis*, commonly known as the glasshouse and lupin biotypes, that have the same identification features but are associated with slightly different climates (hot/dry versus cool/ moist climates) (Brunner & Frey, 2010; Rugman-Jones *et al.*, 2010). There are sympatric populations in the USA,

New Zealand and China, but the glasshouse biotype (preferring hot/dry conditions) has spread most widely and is considered the most damaging (Brunner & Frey, 2010; Rugman-Jones *et al.*, 2010). The key to its success in colonising new areas is its adaptable and polyphagous nature, and field populations vary in their body size (Kirk, 1990), colour (Bryan & Smith, 1956), resistance to insecticides (Jensen, 2000), virus transmission (van de Wetering *et al.*, 1999), life history parameters (de Kogel *et al.*, 1997) and even in response to kairomones (W.J. de Kogel, pers. comm., 2011). *Frankliniella occidentalis* is most attracted to flowering plants as pollen is their preferred food source (Trichilo & Leigh, 1988). It has been recorded on over 240 host-plant species from 62 different families (Tommasini & Maini, 1995), but also feeds on small invertebrates such as eggs of spider mite, *Tetranychus urticae* (Trichilo & Leigh, 1986).

Frankliniella occidentalis was first recorded in the UK in 1986 (Baker *et al.*, 1993), since when it has established in and around commercial greenhouses throughout the country and has become a major pest of glasshouse crops such as cucumber, pepper and chrysanthemum (Kirk, 2002). It can survive outside in southern Britain but its range is predicted to spread northwards with global warming (Cannon, 2004). *Frankliniella occidentalis* was not recorded on UK strawberry in 1990 (Easterbrook, 1991), but incidence in soft fruit crops has increased with the use of semi-protected (open-sided) polytunnels, which now cover about 80% of UK strawberry (Garthwaite *et al.*, 2013). Polytunnels are used to improve fruit quality and extend the growing season, but they also provide a more suitable environment for *F. occidentalis* survival and development. *Frankliniella occidentalis* is currently the most damaging pest of semi-protected strawberry in the UK (Cross, 2012).

Frankliniella occidentalis has six stages in its life cycle: egg, two larval stages (first and second-instar larvae), two pupal stages (propupa and pupa) and adult. Adult females make a slit into plant tissue with their ovipositor and lay their kidney-shaped eggs singly into leaves, sepals, flowers and fruit. Eggs hatch into the pale-coloured wingless larval stages, which are active feeders and are usually found hidden inside flowers or terminal buds (Lewis, 1973). Most larvae drop to the ground to pupate in the soil or leaf litter (Holmes *et al.*, 2012), but a greater proportion stay on plants with dense foliage (Broadbent *et al.*, 2003), complex flowers (Buitenhuis & Shipp, 2008), or when relative humidity (RH) exceeds 81% (Steiner *et al.*, 2010). The two pupal stages are white and have wing buds, but are non-feeding and sessile (Moritz, 1997). On hatching, the winged adults aggregate

to feed and mate (Terry & Gardner, 1990). Females are larger and more variable in colour than males, which are uniformly pale (Bryan & Smith, 1956).

Frankliniella occidentalis has an unusual haplodiploidy reproduction, which contributes to its success as a crop pest. Fertilised eggs are diploid and produce females and unfertilised eggs are haploid, producing males (arrhenotoky) (Moritz, 1997). As females do not have to mate to lay eggs, new colonies can develop from individual females, aiding spread and population increase. The development of pesticide-resistant strains is accelerated as recessive, resistant genes would be expressed in haploid males, which would survive pesticide treatments and breed with other related survivors. The sex ratio reflects the population density, with a higher proportion of males at low pest densities and more females at higher pest densities (Higgins & Myers, 1992), although there is usually a female bias as females live longer than males. The sex ratio is also influenced by dispersal (females disperse earlier than males), attraction to colour, time of day, plant distribution, host quality, age of the female (the proportion of daughters decreases as the females age) and temperature (the proportion of daughters increases with temperature) (Matteson & Terry, 1992; Gaum *et al.*, 1994; Kumm & Moritz, 2010).

Under suitable conditions, *F. occidentalis* populations can increase rapidly in crops. It has an intrinsic rate of increase (r_m) of 0.17 per day on cucumber leaves at 25°C (van Rijn *et al.*, 1995). Development time decreases in a linear relationship with temperatures between about 10 and 35°C, above a minimum temperature for development of about 8°C (McDonald *et al.*, 1998). There is no obligate diapause in *F. occidentalis*, which breeds throughout the year under suitable conditions (Ishida *et al.*, 2003). Overwinter temperatures are critical to survival and early-season development and these are discussed with reference to UK strawberry in Chapter 3. Development rates are highly variable, being affected by plant quality, host plant species (Zhang *et al.*, 2007), cultivar (Rahman *et al.*, 2010) and daylength (Whittaker & Kirk, 2004). On strawberry, egg to adult development time decreased from 33 days at 16°C to about 10 days at 31°C (Nondillo *et al.*, 2008). The presence of pollen increases longevity and fecundity (Trichilo & Leigh, 1988), so the flowering pattern within a crop can have a great effect on phenology in the field. Adult females lay eggs throughout their lives, laying about two eggs per day when fed on strawberry leaves and about 7 eggs per day when fed on strawberry flowers, while living for 13 and 15 days on leaves and flowers respectively (25°C, cv. Aromas) (Nondillo

et al., 2009). In laboratory cultures, females can live up to 75 days and lay over 200 eggs (Robb & Parrella, 1991), but the longevity on different host plants in the field is unknown.

Frankliniella occidentalis is most active during the day, although limited flight, walking, pollen consumption and oviposition occurs during the night (O'Leary & Kirk, 2004; Whittaker & Kirk, 2004). Flight is more frequent above light intensities of 7 – 14 Wm^{-2} and is temperature dependent, with no take-off at 15°C and increasing take-off between 20 and 30°C (O'Leary, 2005). As a result, peak flight occurs around the middle of the day in North European greenhouses when temperatures are warmest (Kiers *et al.*, 2000), although a dip is observed in the mid-day heat when temperatures exceed about 30°C (Mateus, 1998). Flight is concentrated near or just above the top of the crop, where most flowers and new growth occurs (Shipp & Zariffa, 1991). Flight speed of *F. occidentalis* is estimated at 10-22 cm per second (Mateus, 1998) and thrips land at windspeeds above this (Teulon *et al.*, 1999; Pearsall, 2002). Thrips take off to find new hosts when they are starved (Liang *et al.*, 2010), at higher densities (O'Leary, 2005) and when a crop has been harvested or has senesced (Lewis, 1964). Female *F. occidentalis* have a greater dispersal response to flower senescence than males (Rhainds & Shipp, 2003), possibly because they require higher quality food for egg-production. In outdoor crops, wind-speed is often stronger than thrips flight-speed and thrips may drift long distances, carried on wind currents. The related *Frankliniella tritici* migrates annually to infest strawberry fields in north-eastern USA where they have not overwintered, carried on spring frontal systems (Stannard, 1968), but the distance travelled by *F. occidentalis* between UK fields is unknown. Flight is more frequent and prolonged at higher humidities (Terry & Gardner, 1990; Liang *et al.*, 2010) and swarming is sometimes observed before storms, partly in response to changes in barometric pressure (O'Leary, 2005; Kirk, 2007). Thrips are known to land in response to attractive colours and scents (Brødsgaard, 1990), but it is not known what distance they can fly towards an attractive source. Further information on flight behaviour would help to improve trapping programmes.

Colours and scents are used by flower-inhabiting thrips to locate flowers (Kirk, 1984; Terry, 1997) and both are utilised to increase trap catch for the monitoring or control of thrips. *Frankliniella occidentalis* are most attracted to blue, violet, white and yellow traps, which reflect their choice of flower colour (Brødsgaard, 1989; Robb, 1989). A specific shade of blue, with a peak reflectance at 450 nm, is the most attractive in greenhouse-grown crops (Brødsgaard, 1989; Vernon & Gillespie, 1990; Matteson *et al.*, 1992),

although the reason is not known. Attraction increases with light intensity and hue brightness (Matteson & Terry, 1992) but reduces with increased ultraviolet (UV) reflectance above 35% (Matteson *et al.*, 1992; Walbank, 1996). Males show a greater response to attractive colours than females when they are swarming (Vernon & Gillespie, 1990; Matteson & Terry, 1992).

Polyphagous thrips species, such as *F. occidentalis*, are attracted to odours that are common to many different flower species and they are more attracted to scented flowers than odourless ones (Annand, 1926). Over 1,700 compounds have been identified from flowers (Knudsen *et al.*, 2006) of which at least 45 are reported as thrips attractants, including benzenoids, terpenes and nitrogen-containing compounds (Koschier *et al.*, 2000; Koschier, 2008; Davidson *et al.*, 2008). The most promising compounds have been patented and are sold commercially to improve trap catch, either for monitoring or control (Table 1.1). The increase in *F. occidentalis* trap catch with scents can be relatively small (e.g. $\times 2$ -3) for resident thrips and the interpretation of trap catches requires an understanding of the behavioural response of the thrips species to each odour (e.g. increased activity or landing) and their movement within and between crops (Kirk, 1985c; Davidson & Teulon, 2007) (see Chapter 5). The greater increase in trap catch observed to odours in *Thrips obscuratus* (e.g. $> \times 1000$) is partly explained by the thrips behaviour, as it migrates into peach orchards from surrounding areas in response to scents from ripening peaches (El-Sayed *et al.*, 2014). The response to scent is reduced when in competition with other scents from the crop or surrounding vegetation (Davidson *et al.*, 2009). The colour and size of traps is important as the odours are less effective without a colour component (Kirk, 1987; Teulon *et al.*, 1999). Some scents, such as carvacrol, thymol and *cis*-jasmone, are repellent and reduce feeding damage, so have potential for crop protection (Koschier, 2008; Egger & Koschier, 2014). The use of scented and coloured traps for monitoring and control of *F. occidentalis* is discussed further in Chapters 5 and 6.

Pheromones are used widely in crop protection, especially against lepidopteran and coleopteran pests, mostly for monitoring, mass trapping, mating disruption and improving pesticide efficacy (Howse, 1998). The discovery of pheromones in thrips is relatively recent and the role of pheromones in defensive, aggregation and mating behaviour is still being explored and has yet to be fully exploited in crop protection. An alarm pheromone found in the anal droplets of *F. occidentalis* larvae, containing decyl acetate and dodecyl acetate, repels and reduces oviposition rate in adults and increases larval activity (Teerling

et al., 1993b; MacDonald *et al.*, 2003). It causes larvae to jerk and wag their abdomens at predators and drop off plants (van der Hoeven & van Rijn, 1990) and could be used to enhance pesticide efficacy (Cook *et al.*, 2002) or attract natural enemies such as *Orius* spp. (Teerling *et al.*, 1993a). Male adult *F. occidentalis* produce an aggregation pheromone, neryl (*S*)-2-methylbutanoate, which attracts both males and females (Hamilton *et al.*, 2005) and has been used to increase trap-catch (Table 1.1). Males aggregate in prominent or distinctive areas (lek-like) where females are likely to be found (Terry & Gardner, 1990), typically in the most visible (often top) flowers in a crop (Terry & DeGrandi-Hoffman, 1988; Sampson & Kirk, 2012). They defend small territories by lining up side by side, wagging and flicking their abdomens (Terry & Dyreson, 1996; Olaniran & Kirk, 2012). Females enter the aggregation to mate and then leave again (Terry & Gardner, 1990). There is a period of calm during copulation, which could involve pheromones (Pelikan, 1951; Terry & Schneider, 1993). Olaniran (2013) observed the same behaviour by females in response to lavandulyl acetate, suggesting that it may be involved. In contradiction, Zhu *et al.* (2012) consider lavandulyl acetate to be part of the aggregation pheromone but did not test this theory with synthetic compounds. A newly identified contact pheromone, 7-methyltricosane, causes increased activity in males and causes females to raise their abdomens to reject mating (Olaniran *et al.*, 2013). In order to gain further insight into the role of these compounds and their possible use in pest management, various combinations of the synthetic compounds were field-tested in this study (see Chapter 5).

1.2. Pest status of *Frankliniella occidentalis*

The world-wide pest status of *F. occidentalis* reflects the wide range of crops from different plant families, continents and sectors that it attacks. Host plants include outdoor-grown crops, such as cotton, top fruit, peanuts, tomato, lettuce, peas, onion and grape, and greenhouse-grown crops, such as cucumber, sweet pepper, strawberry, chrysanthemum and rose (Robb, 1989; Terry, 1991; Frey, 1993; Leigh, 1995). Both adult and larval *F. occidentalis* cause direct damage by feeding on plant leaves, flowers and fruit (Childers, 1997). Symptoms include leaf scarring, spotting, necrosis, distorted growth and deformed flowers (Kirk, 1997a). Blotches of silvering is a typical symptom on leaves and petals, caused by air once fluid has been sucked out of the plant, which may also be spotted by dark faecal deposits (Mound, 1971). Feeding often results in distorted growth when thrips

feed on young tissue, damaging cells which collapse and fail to expand during growth. For example, a small amount of feeding on young cucumber fruit results in curling or pigtailling (Jacobson, 1997). Uncontrolled, *F. occidentalis* feeding can result in direct crop loss or unmarketable produce as well as downgraded plants sold at a lower price (Shipp *et al.*, 1998). On strawberry, the most important damage caused by *F. occidentalis* feeding is fruit bronzing and weight loss, but the damage and tolerance to damage varies considerably between cultivars, climate and markets (Steiner & Goodwin, 2005a; Coll *et al.*, 2007a), so local knowledge is required. The relationship between thrips density and fruit damage was quantified under UK conditions in this study (see Chapter 4).

As well as causing feeding damage, *Frankliniella occidentalis* is an important vector of tospoviruses such as *Tomato spotted wilt virus* (Tospovirus; Bunyaviridae TSWV) and can spread bacterial and fungal diseases which enter plants damaged by thrips feeding (Ullman *et al.*, 1997). Strawberry is not known to be a host of any thrips-vectored viruses (Parrella *et al.*, 2003a), but *F. occidentalis* can exacerbate the spread of *Botrytis cinerea* (Coll *et al.*, 2007a).

Quantification of global crop losses due to *F. occidentalis* is difficult because of the diversity of areas and crops in which damage occurs (Kirk, 2002). In the Netherlands alone, annual losses from direct damage were estimated at \$30 million, with a further \$19 million due to TSWV when *F. occidentalis* first arrived in the country (Roosjen *et al.*, 1998). Globally, annual losses from TSWV during the early 1990s exceeded \$1 billion (Goldbach & Peters, 1994). Further costs have been incurred by countries and growers in trying to eradicate *F. occidentalis* when it was considered a quarantine pest. For example, Finland spent US\$390,000 between 1987 and 1990 on attempted eradication (Rautapää, 1992). The losses due to *F. occidentalis* change annually as its geographical range continues to expand, resulting in more damage in some crops and regions, while the implementation of successful IPM programmes in other crops and regions has improved control and reduced damage (Sampson *et al.*, 2009). In semi-protected UK strawberry crops, growers have suffered increasing losses to *F. occidentalis* over the last decade, as pesticide-resistant strains have spread through strawberry-growing regions and annual UK losses are estimated at £7-11 million (see Chapter 4).

1.3. Management of *Frankliniella occidentalis*

In protected crops in Northern Europe, the most successful and sustainable control programmes rely on the integration of all appropriate control methods including natural enemies (van Lenteren, 2007), commonly known as Integrated Pest Management (IPM) (Dent, 1995). The adoption of successful IPM worldwide has resulted in a significant reduction in pesticide use (Baker *et al.*, 2002) with resulting benefits for human health as well as improved, more sustainable control (Peshin *et al.*, 2009).

The main driver for the adoption of IPM by UK strawberry growers is the limited number of insecticides that are registered for use against *F. occidentalis* on strawberry (Table 1.2) and the poor control often achieved with those that are available. This has resulted in complete crop loss on some occasions (R. Harden, pers. comm., 2013) and alternative control methods are sought. Poor control can be the result of insecticide-resistant biotypes, which are widespread globally following indiscriminate use of insecticides (Immaraju *et al.*, 1992; Jensen, 2000; Bielza, 2008; Sparks *et al.*, 2012). In consequence, insecticides are used increasingly in combination with natural enemies, so their compatibility must be considered (Table 1.2). Spinosad (Tracer, Landseer, Chelmsford, UK) is most commonly used as it can be highly effective against *F. occidentalis* (Rahman *et al.*, 2011b). It has low mammalian toxicity and its use can be integrated with predatory mites on strawberry (Rahman *et al.*, 2012). However, spinosad-resistant *F. occidentalis* populations were found within three years of its commercial release in the UK (Colin Cater, Landseer, pers. comm., 2011) and are widespread throughout the world (Sparks *et al.*, 2012). Resistant thrips populations compete well in the field, but tend to get diluted by more susceptible biotypes at the end of the season (Contreras *et al.*, 2008), so some efficacy can be retained by minimising insecticide use and rotating active ingredients from different chemical groups as a resistance management strategy (Denholm & Jespersen, 1998; Bielza, 2008). More harmful insecticides with a residual action of several weeks cannot be integrated easily with natural enemies unless separated by time (e.g. used as end of season clean-up) or space (e.g. used as spot-treatments or soil treatment). With few chemical options available, the use of biopesticides and physically acting products offer possible alternatives.

Following a change in EU pesticide regulations aimed at improving food safety, fewer chemical insecticides are likely to be available to growers (Parente, 2006), so it essential to

make the best use of those that are available. Control failure can be the result of poor spraying technique and timing rather than resistance, as *F. occidentalis* is difficult to target and the strawberry canopy difficult to penetrate. Spray equipment and technique can be manipulated to improve thrips control (Lewis, 1997c). A small droplet size and high spray pressure is required to penetrate the dense canopy of leaves in strawberry (Cross *et al.*, 2000). Increased water volume, electrostatic spraying, adjuvants and cultural methods such as de-leafing could be used to improve penetration of the canopy (Helyer & Brobyn, 1992; Seaton *et al.*, 1997). Sprays are usually most effective at times of the day when thrips are most active as they are more exposed, but the use of sugars or the alarm pheromone have been found to increase activity and thereby improve pesticide efficacy (Cook *et al.*, 2002; Parrella *et al.*, 2003b).

Cultural methods such as the choice of cultivar, growing methods and crop hygiene play an important role in IPM. Starting the season with low pest numbers allows time to establish other control measures, such as predators and traps, before thrips numbers build up. Incorporating hygiene measures into the normal routine of a farm, including weeding and end of season clean-ups to remove infested plant material and inspecting incoming plant material for thrips makes a vital contribution to thrips control (Parrella, 1996). Weed control is especially important between crops as they provide a green bridge to carry thrips populations over to new or second-year crops (Katayama, 2006). Flowering plants, such as chrysanthemum or verbena, have been identified that are very attractive to thrips, drawing them away from the crop as a trap crop, which can then be sprayed or removed (Buitenhuis *et al.*, 2007), although whether these plant species would compete with a flowering strawberry crop is unknown. As flowering plants in field margins are also refuges for natural enemies (Atakan, 2010), further work is required to identify field margins that minimise thrips invasion yet maximise naturally occurring beneficial insects for pollination and pest control (Wackers *et al.*, 2008). The advantages of growing strawberry over one, two or three seasons has to be weighed against the increasing thrips populations that can accumulate with each successive season. As climate, alternative hosts and growing systems affect *F. occidentalis* populations, some basic information was required on its phenology in semi-protected UK strawberry crops to help target possible IPM methods (see Chapter 3).

The choice of cladding and bed mulch can affect thrips numbers in polytunnels. Plastic films that block ultraviolet (UV) transmission (200-400 nm) but allow visible light

(400-700 nm) can result in 50-80% reduction in thrips (Antignus *et al.*, 1996; Doukas & Payne, 2007). Reflective materials, such as aluminiumised tape, are repellent to *F. occidentalis* and can reduce thrips invasion into closed polytunnels by 55% when placed around the entrances (McIntyre *et al.*, 1996). Reflective sheets have also been used as a mulch, reducing thrips populations by 33-68% in vegetable and ornamental crops (Greenough *et al.*, 1990; Csizinszyk *et al.*, 1995), although the effects reduce as soon as the crop canopy shades the mulch. The use of plastic skirts as a physical barrier can delay the spread of thrips between fields with different infestation levels as thrips are generally low flying (Yudin *et al.*, 1991).

Physical, chemical and phenotypic traits of plants, such as trichome length, phenylpropanoid levels and flower colour can confer varietal resistance or tolerance to *F. occidentalis* in different crops (Kumar *et al.*, 1995; Soria & Mollema, 1995; Maris *et al.*, 2004; Leiss *et al.*, 2009). On strawberry, cultivar differences have been observed in susceptibility to damage and tolerance to *F. occidentalis*, for example, females produced fewer live young, had a longer development time and reduced longevity on the cultivar ‘Albion’ compared to ‘Camarosa’ (Rahman *et al.*, 2010). The timing and abundance of flowering can be controlled by planting and cladding dates and by de-flowering, which could also be used to manage thrips populations, as flower availability is a key factor in *F. occidentalis* population build-up (Gerin *et al.*, 1999).

The temperature and humidity cannot be manipulated in polytunnels in the same way as glasshouse crops, but some physical manipulation may be possible. Watering and fertilisation regimes can affect thrips populations (Schuch *et al.*, 1998) and damage symptoms (Larson *et al.*, 2004). In the 2011 season, UK strawberry growers were trialing the use of hot air and vacuum treatments previously used against capsids (Pickel *et al.*, 1994), but further work is required to refine heat treatments so that they do not scorch the strawberries or disrupt natural enemies.

Natural enemies are an essential part of managing pesticide-resistant *F. occidentalis* in strawberry (Coll *et al.*, 2005; Steiner & Goodwin, 2006; Shakya *et al.*, 2010; Rahman *et al.*, 2011a; Sampson *et al.*, 2011). Over 300 natural enemy species of *F. occidentalis* have been identified and evaluated in different crops around the world including predators (Riudavets, 1995), parasitoids (Loomans & van Lenteren, 1995), nematodes (Loomans *et al.*, 1997) and entomopathogenic fungi (Butt & Brownbridge, 1997). In practice, relatively

few species are released commercially, constrained by the need for economic mass rearing, start-up costs and legislation (van Lenteren *et al.*, 2006) (Table 1.3). Naturally occurring species, such as *Orius* spp., can be sufficient to control *F. occidentalis* without inundative releases where broad-spectrum pesticides are avoided (Coll *et al.*, 2007a).

The predatory mite *Neoseiulus cucumeris* (formerly *Amblyseius cucumeris*) is the most widely used predator against *F. occidentalis* in UK strawberry crops (Garthwaite *et al.*, 2013). These small predatory mites only feed on eggs and first instar larvae (Shipp & Whitfield, 1991), so control relies on inundation of the crop with predators before thrips adult numbers build up (Jacobson *et al.*, 2001b). *Neoseiulus cucumeris* can establish in the absence of thrips in pollen-producing crops, but the frequency and numbers required to prevent crop damage in semi-protected strawberry needs further quantification (Fitzgerald & Jay, 2011). Environmental conditions are critical for establishment as *N. cucumeris* is active between 8-30°C and eggs die below 65% RH (van Houten & van Lier, 1995; Shipp & van Houten, 1997). Control failure may result from releasing too few predators too late, from invasions of adult thrips (Shipp & Whitfield, 1991), or from the use of incompatible pesticides against thrips or other pests (Malezieux *et al.*, 1992). As *N. cucumeris* is the main predator used in semi-protected strawberry in the East Midlands, UK, it was considered as a factor in the economic injury levels defined in Chapter 4.

The soil dwelling mites *Stratiolaelaps scimitus* (formerly *Hypoaspis miles*) and *Macrocheles robustulus* and the predatory ground beetle *Dalotia coriaria* (formerly *Atheta coriaria*) can be used to reduce pupal stages of *F. occidentalis* (Bennison, 2006; Messelink & Van Holstein-Saj, 2008). The combined use of predators in the soil and on the plant results in a more robust control strategy as the different predators attack different thrips stages. Also, soil predators are more protected from insecticide treatments than plant predators (Wiethoff *et al.*, 2004). *Stratiolaelaps scimitus* is commonly used in combination with *N. cucumeris* in glasshouse strawberry and there is evidence that it would be equally effective in tunnel-grown strawberry (Rahman *et al.*, 2011a).

The predatory bugs *Orius* spp. are widely used in combination with predatory mites as they are voracious polyphagous predators that feed on both adult and larval *F. occidentalis* on strawberry (Shakya *et al.*, 2010). *Orius* spp. can be very effective on strawberry in Southern Europe and *F. occidentalis* populations decline sharply once flower occupation is high (Coll *et al.*, 2005; Sampson *et al.*, 2011). *Orius laevigatus* is used in protected crops

in north European crops as it has a shorter diapause than *O. majusculus*, so can be released earlier in the season (Chambers *et al.*, 1993). Cool temperatures and a relatively long generation time (egg to egg takes 3-4 weeks at 25-20°C (Alauzet *et al.*, 1994)) can limit its use in UK strawberry as mortality is high below 15°C and egg-laying mainly occurs at temperatures between 20-30°C (Riudavets *et al.*, 1995). As a result, *Orius* spp. populations may not build up fast enough to prevent thrips outbreaks unless released in large (uneconomic) quantities. Improved early season establishment is sought by timing releases to correspond with flowering or by the addition of banker plants with pollen to boost food, especially between flower flushes (Boullenger *et al.*, 2010; Bennison *et al.*, 2011), a technique that has been used effectively in cut roses (Bueno *et al.*, 2009). New rearing methods are being investigated that may reduce the cost of *Orius* spp., which could make higher release rates affordable (Arijs & De Clercq, 2001; Mendes *et al.*, 2005).

A second line of defence may be required to correct predator: prey imbalance and there are several biological options available which can be applied through conventional spray equipment and would offer a safer option than chemical insecticides. *Frankliniella occidentalis* is susceptible to at least seven species of entomopathogenic fungi (Brownbridge *et al.*, 2000; Ansari *et al.*, 2008). *Lecanicillium* spp. require high humidities (Helyer *et al.*, 1992) and are unlikely to work on strawberry foliage although could be used in the soil (Sermann *et al.*, 1996). *Beauveria bassiana* (Naturalis-L, Intrachem, Italy) is more tolerant of lower humidities (Shipp *et al.*, 2003) and achieved control equivalent to that of chemicals (65-87%) in cucumber (Jacobson *et al.*, 2001a), but efficacy has not been demonstrated in strawberry. *Metarhizium anisopliae* (Met 52, Fargro) has resulted in useful control (53-75%) of pupae in soil or compost (Brownbridge *et al.*, 2011). Novel methods of applying *M. anisopliae* are being developed, using “attract and infect” traps (Niassy *et al.*, 2012), which could be tested in UK crops. Entomopathogenic nematode species, such as *Steinernema feltiae*, can reduce adult and pupal stages of *F. occidentalis*, although control relies on repeated applications and sufficient moisture for the nematodes to locate their hosts before drying out (Ebssa *et al.*, 2004; Buitenhuis & Shipp, 2005).

Many natural enemy species have been identified that are specific to thrips and could offer exciting opportunities for improved control in the future. These include the nematode *Thripinema nicklewoodi* (Greene & Parrella, 1995), the Entomophthorales fungi *Entomophthora thripidum* (Samson *et al.*, 1979) and *Neozygites parvispora* (Ananthakrishnan, 1993). Economic mass production of these potentially important

control agents is the key to getting them to market, offering a challenge to research workers.

The implementation of IPM relies on accurate methods of estimating population density to determine the timing of preventative treatments, the success of existing treatments or the need for remedial treatments. This is critical in the control of pesticide-resistant *F. occidentalis* so that the use of unnecessary insecticide treatments can be avoided, as these may harm predators and increase resistance levels (Denholm & Jespersen, 1998). Counts of thrips in flowers are most commonly used to estimate thrips density in strawberry, as it is a cost-effective method that can be used by growers (González-Zamora & Garcia-Marí, 2003), although traps can also be used (Steiner & Goodwin, 2005b). To interpret monitoring results, growers need to know what density of thrips is likely to result in economic crop loss. Published action, economic injury and damage thresholds are very variable in strawberry, ranging from 3-24 thrips per flower and there is a need to determine economic injury levels under UK conditions because thresholds vary with the cultivar, local market price and damage tolerance (see Chapter 4).

1.4. Aims of the study

This study was part of a larger project carried out in collaboration with science partners in the UK (ADAS, EMR, Warwick HRI and NRI). The overall aim was to develop a comprehensive range of new effective methods for managing insecticide-resistant *F. occidentalis* on semi-protected strawberry in the UK. The methods investigated by other partners included a computer-based population and risk forecasting model, new selective pesticide treatments, new biopesticides and novel, more cost-effective strategies for using existing predators. These components were to be integrated with the monitoring and trapping methods developed in this study in order to recommend a comprehensive management strategy for the pest.

The overall aim of this study was to improve the management of *F. occidentalis* in semi-protected strawberry in the UK, by developing an easy to use monitoring method with attendant economic injury levels (EILs), based on new insight into thrips biology, and by investigating the viability of using traps for control of *F. occidentalis*.

The specific objectives were to:

- (1) develop an easy to use monitoring method for thrips for use by growers on strawberry (see Chapters 2 and 4);
- (2) observe the phenology of *F. occidentalis* in UK strawberry fields (see Chapter 3);
- (3) quantify the damage to strawberry fruit caused by *F. occidentalis* (see Chapter 4);
- (4) determine flower count EILs for *F. occidentalis* in semi-protected strawberry (see Chapter 4);
- (5) optimise pheromone use for the monitoring and trapping of *F. occidentalis* (see Chapter 5);
- (6) develop a practical method for mass trapping (see Chapter 6);
- (7) test whether mass trapping of *F. occidentalis* reduces crop damage and is economically viable (see Chapter 6).

Table 1.1. Some odours that increased trap catches of thrips compared to unbaited controls.

| Odour | Trap colour | Trap increase | Species | Reference |
|--------------------------------------|-------------|---------------|--------------------------|--------------------------------|
| Benzaldehyde | White | ×4 | <i>T. tabaci</i> | Teulon <i>et al.</i> , 2007b |
| <i>p</i> -anisaldehyde | White | ×3-8 | <i>Thrips</i> spp. | Kirk, 1985c |
| | White | ×2-3 | <i>T. imaginis</i> | Kirk, 1987 |
| | Yellow | ×2-6 | <i>F. occidentalis</i> | Teulon <i>et al.</i> , 1993 |
| | Yellow | ×10 | <i>F. occidentalis</i> | Hollister <i>et al.</i> , 1995 |
| | Blue | ×2 | <i>F. occidentalis</i> | Brødsgaard, 1990 |
| | Blue | ×2 | <i>F. occidentalis</i> | Frey <i>et al.</i> , 1994 |
| | Yellow | ×11-15 | ♀ <i>F. occidentalis</i> | Teulon <i>et al.</i> , 1999 |
| | Yellow | ×3-20 | ♂ <i>F. occidentalis</i> | Teulon <i>et al.</i> , 1999 |
| | White | ×4 | <i>T. tabaci</i> | Teulon <i>et al.</i> , 2007b |
| Ethyl nicotinate | Beige | >100 | <i>T. obscuratus</i> | Penman <i>et al.</i> , 1982 |
| | White | ×27 | <i>T. obscuratus</i> | Teulon <i>et al.</i> , 1993 |
| | Blue | ×2 | <i>F. occidentalis</i> | Frey <i>et al.</i> , 1994 |
| | White | >100 | <i>T. obscuratus</i> | Teulon <i>et al.</i> , 2007b |
| | White | ×4 | <i>T. tabaci</i> | Teulon <i>et al.</i> , 2007b |
| | Yellow | ×0 | <i>F. occidentalis</i> | Davidson <i>et al.</i> , 2007 |
| Ethyl isonicotinate | White | ×31 | <i>T. tabaci</i> | Teulon <i>et al.</i> , 2007b |
| | Yellow | ×14 | ♀ <i>F. occidentalis</i> | Davidson <i>et al.</i> , 2007 |
| Geraniol | Blue | ×2 | <i>F. occidentalis</i> | Frey <i>et al.</i> , 1994 |
| Methyl isonicotinate | Yellow | ×14 | ♀ <i>F. occidentalis</i> | Davidson <i>et al.</i> , 2007 |
| | Yellow | ×5 | ♂ <i>F. occidentalis</i> | Davidson <i>et al.</i> , 2007 |
| | White | ×9 | <i>F. occidentalis</i> | Davidson <i>et al.</i> , 2009 |
| Methyl anthranilate | Green | ×3-50 | <i>T. hawaiiensis</i> | Murai <i>et al.</i> , 2000 |
| 6-Pentyl-2H-pyran-2-one | Red | >1000 | <i>T. obscuratus</i> | El-Sayed <i>et al.</i> , 2014 |
| Neryl (<i>S</i>)-2-methylbutanoate | Blue | ×3 | <i>F. occidentalis</i> | Gómez <i>et al.</i> , 2006 |
| | Yellow | ×2-3 | <i>F. occidentalis</i> | Broughton, 2009 |
| | Blue | ×2 | <i>F. occidentalis</i> | Sampson & Kirk, 2013 |

Table 1.2. Chemical and physical pesticides with label or off-label approval for use on semi-protected strawberry crops in the UK, 2013 (Lainsbury, 2013), which may have some activity against thrips. The compatibility of selected pesticides with *Neoseiulus cucumeris* is taken from the Koppert side-effects list (Koppert, 2014) unless stated otherwise, where harmful >75% mortality following direct contact. The side-effects lists provided by commercial biological control companies are compiled partly from IOBC (International Organisation of Biological Control) side-effects testing programme, as well as published papers and field experience.

| Product | Active ingredient | Compatibility with <i>N. cucumeris</i> | Comments |
|--|-------------------|--|--|
| Decis (Bayer cropscience) | deltamethrin | Harmful for >8 weeks | Use should be avoided during the growing season |
| Equity (Dow) | chlorpyrifos | Harmful for >6 weeks | Use should be avoided during the growing season. |
| Pyrethrum 5 EC (Agropharm) Spruzit (Certis) | pyrethrins | Harmful for <1 week | Poor thrips control. Predators can be re-released after treatment. |
| Majestik (Certis) | maltodextrin | Harmful for 1 day ^a | Physically acting, must achieve good contact. |
| Savona (Koppert) | Fatty acids | Harmful for 1 day ^a | Physically acting, must achieve good contact. |
| Tracer (Lanseer) | spinosad | Harmful for <1 week ^b | Resistant populations of thrips occur. Can be integrated with predators. |
| Dynamec (Syngenta Bioline) | abamectin | Harmful for 2 weeks ^c | Low-moderate thrips control. Residual effect is shorter during the summer. |

^a No residual effect once dry.

^b Data from (Rahman *et al.*, 2011b).

^c The residual effect is shorter in the summer as the chemical is degraded by sunlight.

Table 1.3. Commercially available natural enemies of *F. occidentalis* for release in semi-protected strawberry crops in the UK, 2013.

| Phylum/Order | Species | References |
|--------------|----------------------------------|--------------------------------|
| Hemiptera | <i>Orius laevigatus</i> * | Tomassini & Nicoli, 1995 |
| | <i>Orius majusculus</i> | Tomassini & Nicoli, 1995 |
| Coleoptera | <i>Dalotia coriaria</i> | Bennison, 2006 |
| Mesostigmata | <i>Stratiolaelaps scimitus</i> * | Berndt <i>et al.</i> , 2004 |
| | <i>Geolaelaps aculeifa</i> | Gillespie & Ramey, 1988 |
| | <i>Neoseiulus cucumeris</i> * | Bennison <i>et al.</i> , 2002 |
| | <i>Macrocheles robustus</i> | Messelink <i>et al.</i> , 2006 |
| Nematoda | <i>Heterorhabditis megidis</i> | Greene & Parrella, 1995 |
| | <i>Steinernema feltiae</i> | Buitenhuis & Shipp, 2005 |
| | <i>S. carpocapsae</i> | Bennison <i>et al.</i> , 2007 |
| Hyphomycetes | <i>Beauveria bassiana</i> | Wright & Kennedy, 1996 |
| | <i>Metarhizium anisopliae</i> | Ansari <i>et al.</i> , 2007 |

Source: Product lists from Biocontrol suppliers in the UK: Certis UK, Biobest, Fargo, Koppert, Syngenta Bioline. Note: Some generalist predators that may feed on a few thrips but are not released specifically for their control, such as *Amblyseius andersoni*, *Neoseiulus californicus*, and *Adalia* spp. have been excluded.

* = The species most commonly released in UK strawberry

Chapter 2

General Methods

2.1. Introduction

General methods that were common to several experiments are included here. The site details and growing methods are summarised for the UK strawberry (*Fragaria x ananassa*) trial sites. Methods of trapping, rearing, sampling, collecting and identifying thrips are shown, as well as methods for collecting temperature and humidity data. Statistical methods were standardised throughout the study. Further details or anomalies that are specific to individual experiments are given within the chapters where they are reported.

2.2. Site details and growing systems

With the exception of the pheromone screening experiments that were carried out in Spain (Chapter 5), all field experiments were carried out on three farms in the West Midlands area of the UK from 2011 to 2013. Large, commercial, strawberry growing farms were selected that were 10-40 km apart and had a history of crop loss to *F. occidentalis*:

- Manor Farm (owner, Mr. Simon Clarke), Hinxhill, Tamworth, B78 3DW.
- Littywood Farm (owner, Mr. George Busby), Bradley, Stafford, ST18 9DW.
- New Farm Produce Ltd. (owner, Mr. Stephen McGuffie), Elmhurst, Lichfield, WS13 8EX.

The GPS grid references of field sites are in Table 2.1. All experiments were conducted in semi-protected, everbearer strawberry crops, as these are highly susceptible to thrips damage (R. Harnden, pers. comm., 2011). Everbearer cultivars flower and fruit continuously from about April to October, enabling *F. occidentalis* populations to build up throughout the season. In contrast, main-crop strawberry cultivars only flower for about 60 days and so thrips damage is less common. The cultivars Camarillo (Driscoll's,

Watsonville, USA) and Finesse (East Malling Research, East Malling, UK) were selected for most experiments, as they were most widely grown on the farms used and are susceptible to *F. occidentalis* damage (S. Clarke, pers. comm., 2011). Camarillo was used for controlled damage experiments to enable comparison of data on the same strawberry cultivar between sites and years.

Most commercial UK everbearer crops are grown under open-sided polytunnels during flowering and fruiting. This improves fruit quality and extends the growing season, but also increases the temperature which favours *F. occidentalis* development (McDonald *et al.*, 1998). The polytunnels used in this study were either 8 m wide with five strawberry beds per tunnel or 6.5 m wide with four strawberry beds or rows per tunnel (Figure 2.1 A, B). The tunnels were covered by polythene cladding (Manor farm and New farm produce: Haygrove, 150 micron, 3 season, Haygrove Ltd., Ledbury, UK; Littywood farm: Luminance THB, BPI-Visqueen, London, UK), from approximately early April to late October, and the cladding was removed over winter.

Some crops were grown in raised beds, covered in black plastic mulch, and irrigated by T-tape (Table 2.1, Figure 2.1 A). The planting density was 9.5-10 plants per m². Other crops were grown in coir growbags (10 cm x 100 cm), each contained 10 plants, and placed on a Mypex mulch (Don and Low Ltd, Forfar, UK) with drip irrigation (Dripnet PC, Netafim Ltd, Tel Aviv, Israel) (Table 2.1, Figure 2.1 B). The growbags were spaced to give a density of 10 plants per m². Early season flowers were typically removed from first-year crops during April to increase vegetative growth and strengthen the plant, as is usual commercial practice.

During the experiments, growers continued with their usual pest and disease control programmes. The thrips control mainly consisted of spraying with the insecticide spinosad (Tracer, Landseer Ltd, Chelmsford, UK) and releasing the predatory mite *Neoseiulus cucumeris*. Further details on treatments that are specific to individual experiments are given within the chapters, where relevant.

2.3. Thrips identification

Identification of thrips requires detailed examination of minute features and specimens must usually be cleared, mounted and examined under a high-powered microscope to see

these. The best method for clearing and mounting specimens varies according to the purpose of mounting and length of time required for storage. Museum quality slides, that may last for hundreds of years, are dehydrated in increasing concentration of alcohol, then cleared (e.g. with clove oil) and mounted in Canada Balsam (Kirk, 1996). Aqueous mounts such as polyvinyl lactophenol, Hoyer's medium or Berlese fluid can be used for less permanent mounts (Kirk, 1996). Polyvinyl lactophenol (Harris chemicals, Shenstone, UK) was used in this study, as it clears and sets the thrips well and preserved the slides for at least the length of the study. Although effective, polyvinyl lactophenol is very corrosive and no longer available commercially.

Thrips collected from the field or removed from traps were stored in 70% alcohol (industrial methylated spirits). For identification, adult thrips were mounted on slides and examined under a compound microscope (x 200, Leica ATC 2000, Milton Keynes, UK) using the following procedure:

- One drop (10 µl) of polyvinyl lactophenol (Harris chemicals) was placed on a glass cover slip (13 mm diameter, No. 0, 0.13-0.17 mm thick, Chance Proper Ltd., Waveley, UK) using a micro-pipette.
- A thrips was removed from the alcohol sample using a fine paint brush, then positioned on its back in the polyvinyl in lactophenol on the glass cover slip.
- The thrips abdomen was gently pressed down and the wings spread out with a bent needle, so that it was possible to see the antennae, tergites and wings.
- A glass slide (76 x 26 mm, Thermo Scientific, Menzel-Gläser, Braunschweig, Germany) was placed on the cover slip and flipped over so that the thrips was on its front and bristles on the head could be seen. If the wings had not spread fully, the cover slip was pressed down gently to spread them further, to improve the visibility of key identification features.

Specimens were identified to species using two main keys (Mound *et al.*, 1976; Kirk, 1996). The Royal Entomological Society key (1976) provides thorough identification of the British thrips fauna up to 1976 but does not include some of the invasive pest species that have established or been recorded in the UK since then, such as *F. occidentalis*, *Thrips palmi* and *Echinothrips americanus*. Most of these invasive species are included in Kirk's key (1996), although new species continue to be recorded, particularly in glasshouse crops. Identifications were checked against reference specimens in the collection of W. D. J. Kirk

at Keele University and against photographs in the interactive key of world thrips pests (Moritz *et al.*, 2004). Identification of *F. occidentalis* using molecular methods (RAPD-PCR, random amplified polymorphic DNA-polymerase chain reaction) is possible (Kraus *et al.*, 1999), but this technology was not available commercially during this study (Moritz *et al.*, 2007).

Only three thrips families occur in Britain and the Thripidae were separated from Aeolothripidae by their lack of broad wings and from Phlaeothripidae by their lack of a tube at the end of their body. Within the Thripidae, *Frankliniella* adults were separated from *Thrips* adults by the following characters:

- Antenna comprising of eight distinct segments (Figure 2.2 A).
- Forewing with two complete rows of setae between the hair fringes (Figure 2.2 C).
- A pair of setae anterior to the first ocellus.
- Abdominal tergite 8 with ctenidia anterio-lateral to the spiracles.

Frankliniella occidentalis adults were separated from other UK *Frankliniella* species by the following features:

- The second post ocular setae are long, about 3 x the length of other post-ocular setae (Figure 2.2 B).
- The pronotum anterior setae comprise of two long lateral and four short medial setae.

The three features highlighted in Figure 2.2 were sufficient to identify *F. occidentalis* to species and were usually visible under a stereoscopic microscope (x 50, Wild M5A, Wild AG, Heerbrugg, Switzerland) before mounting. Hence, *F. occidentalis* in alcohol or on sticky traps could be counted under a stereoscopic microscope (Wild M5A, Heerbrugg) without mounting every thrips. Alcohol samples were poured into a petri-dish and the thrips were separated into species or family groups using a fine paint brush before counting. Voucher specimens (over 2000 in total) were selected at random from each group for mounting to confirm the identification, using random numbers and a grid square (5 mm²). Specific details relating to the number of specimens mounted for identification for each experiment are given within the chapters where they occur.

2.4. Counting and removing thrips from traps

Sticky traps removed from field experiments were wrapped in clear plastic and stored in a freezer at -20°C to preserve the thrips. Trap catches of *F. occidentalis* were counted under a stereoscopic microscope (Wild AG, Heerbrugg) in the Keele laboratory, using the identification features detailed above (Figure 2.2). As there were large numbers of thrips on the traps, a transparent acetate sheet with grid lines, 10 mm apart (approximately the field of vision under the microscope), was laid over the top of the trap to aid tracking, and the thrips were tallied using a click counter. To avoid duplication of counts, any thrips falling under a grid line were counted in the cell to the left of the line. Voucher specimens were mounted to confirm the identification. Specific details relating to the number of specimens mounted for identification for each experiment are given in the chapters where they occur.

Where mounting was required to identify a thrips, a small triangular section of the trap containing the thrips was cut out. The section of trap was placed in a small watch glass containing terpentine (White spirit, Fisher Scientific, Loughborough, UK), which dissolved the glue allowing the plastic wrapper to be removed. The thrips was then removed from the trap and mounted on a slide as above (see 2.3).

2.5. Validation of the method used for sampling strawberry flowers

An effective and reliable method of assessing thrips population density was required, that was also rapid and practical so that it could be used by growers for monitoring and decision-making. Estimates of *F. occidentalis* numbers in strawberry usually focus on flowers, where adult thrips are most numerous (García-Mari *et al.*, 1994; Laudonia *et al.*, 2000; González-Zamora & Garcia-Marí, 2003; Steiner & Goodwin, 2005a). The relative accuracy of visual, tap or extraction sampling in flowers varies with the complexity of the flower. Visual counts provide a reasonable estimate of adult thrips in simple flowers, such as busy lizzy (*Impatiens walleriana*), but not in complex flowers, such as marigold (*Tagetes erecta*) (Ugine *et al.*, 2011). Because of the time and expense involved in extraction techniques, visual inspection is considered the most cost-effective sampling method for adult thrips in open flowers like strawberry, recovering about 80% of adults but only 33% of larvae when compared to absolute counts of thrips extracted from flowers

placed in alcohol (González-Zamora & Garcia-Marí, 2003). Differences in *F. occidentalis* abundance and population structure have been observed between flower stages in apple and strawberry (Terry & DeGrandi-Hoffman, 1988; González-Zamora & Garcia-Marí, 2003). Further work was required to test whether the selection of flower stage and flower position for sampling affects *F. occidentalis* population estimates in strawberry.

The aim of this study was to define a reliable method of sampling thrips populations in flowers for the field experiments and for decision-making by growers. The sampling method defined was tested to determine whether it could be used effectively by farm staff, following a short training session. Thus, if reliable, sampling of adult thrips in flowers of a specific age and position, by eye, could be used throughout the study, and these could be related to grower counts used for decision-making.

2.5.1. Flower sampling: materials and methods

2.5.1.1. Which flower should be sampled?

Flower stage. Different stages of flower were sampled in a field of everbearer strawberries (cv. Camarillo) (Table 2.1, field 3) in order to determine the distribution of adults between flower stages. On 5 and 12 July 2011, six stages of flowering were selected for sampling, which could be recognised easily in the field (Table 2.2). On each of the sample dates, 30 plants were selected at random from a 50 m length of strawberry row. One flower from each of the six categories in Table 2.2 was sampled on the selected plants, or the nearest to that plant if there were no flowers of the right stage present. A visual sampling method was used in situ as this was considered the most cost effective method for strawberry growers (González-Zamora & Garcia-Marí, 2003). Each flower was picked and examined carefully using a $\times 7$ optiVISOR head lens (LightCraft, London, UK) while peeling apart the petals to reveal the thrips, and the numbers of adult female, adult male and larval (first and second instar larvae combined) thrips were counted.

Flower position. On 26 July 2011, in the same crop, 27 mature flowers were selected from either the top or the side of randomly selected plants, using random numbers generated by Minitab 16 (Minitab Inc., Pennsylvania, USA), and the numbers of adult female, adult male and larval thrips (first and second instar larvae combined) were counted by eye per flower, using a head lens (as above).

2.5.1.2. Is the sampling method accurate and consistent between samplers?

To test whether the sampling of mature mid-aged flowers, taken from the top of strawberry plants resulted in a consistent estimate of thrips density and could be used by growers, a training hand-out was given to four samplers with instructions on how to count the number of adult thrips per flower. The samplers included two experienced advisors and two inexperienced farm staff. The hand-out showed which age and position of flower to sample and what to count, with pictures of thrips to aid identification (Appendix A). Flowers were taken from the middle of the strawberry beds as these were less disturbed by crop workers and machinery. Two crops were sampled on 6 June 2012, one field with high numbers of thrips (Table 2.1, field 7) and one with low numbers of thrips (Table 2.1, field 3). In each crop, each sampler examined 30 separate flowers by eye, using a x10 hand lens, and recorded the number of adult thrips per flower. At the same time 30 flowers were placed into tubes containing 70% alcohol and the number of thrips counted later under a binocular microscope. The first samples were taken about 15 m in from the open end of the tunnel and samplers moved down the tunnel together so that although different flowers were sampled by each person, they were taken from the same area of crop.

2.5.2. Flower sampling: results

2.5.2.1. Which flower should be sampled?

Flower stage. The abundance and frequency of development stages of *F. occidentalis* were significantly different between the flower stages sampled. Adult females were present in flowers from the white bud stage and there were significantly more females in young and mature flowers than in senescent flowers on 12 July (Kruskal-Wallis test, $H_{(2)} = 13.92$, $P < 0.001$) (Figure 2.3 A). The trend was the same on 5 July although the differences were not statistically significant (Kruskal-Wallis test, $H_{(2)} = 4.07$, $P = 0.13$) (Figure 2.3 B). Adult males colonised open flowers and there were significantly more males in mature and senescent flowers than in young flowers on 5 July (Kruskal-Wallis test, $H_{(2)} = 8.53$, $P = 0.03$) (Figure 2.3 A). On 12 July, the numbers of males were not statistically different between the open flower stages (Kruskal-Wallis test, $H_{(2)} = 2.12$, $P = 0.53$) (Figure 2.3 B), although the proportion of males remaining in senescent flowers compared to peak numbers was 57% compared to 37% of females.

First instar larvae were the only thrips stage found in closed green buds, thereafter larval numbers increased significantly between flower stages as the flowers matured, being most abundant in senescent flowers (Kruskal-Wallis tests, $H_{(2)} = 33.68$, $P < 0.001$ (5 July); $H_{(2)} = 44.03$, $P < 0.001$ (12 July)) (Figure 2.3 A, B). There were significantly more thrips (all stages) in mature and senescent flowers than in young flowers (Kruskal-Wallis tests, $H_{(2)} = 32.24$, $P < 0.001$ (5 July); $H_{(2)} = 36.90$, $P < 0.001$ (12 July)). All thrips stages declined in numbers after flowering.

Mean temperatures for the two weeks before the sample dates were 17.2 °C for 5 July and 18.2 °C for 12 July. The flower development time from the start of white bud to the end of senescence (once all the petals had dropped) was 10-12 days.

Flower position. There were significantly more adult female (Kruskal-Wallis test, $H_{(1)} = 21.4$, $P < 0.001$) and adult male (Kruskal-Wallis test, $H_{(1)} = 14.57$, $P < 0.001$) thrips in mature flowers at the top of the plants than in mature flowers at the side of the plants, but numbers of larvae were not significantly different between flower positions (Kruskal-Wallis test, $H_{(1)} = 0.05$, $P = 0.82$) (Figure 2.4).

2.5.2.2. *Is the sampling method accurate and consistent between samplers?*

Population estimates of thrips were significantly different between samplers in a field with moderate thrips (one-factor ANOVA, $F_{(4, 145)} = 8.2$, $P < 0.001$) but not in a field with low thrips numbers (one-factor ANOVA, $F_{(4, 145)} = 1.48$, $P = 0.2$) (Table 2.3). Tukey's test showed that the population estimates of three of the four samplers were not significantly different from the alcohol samples in the field with moderate thrips population, whereas the population estimate of one sampler (sampler 2, Table 2.3) was lower. Sampler 2 (an experienced advisor) was deliberately sampling thrips numbers quickly as done when crop walking and did not count the numbers of thrips above 10 per flower, which brought down the mean.

2.5.3. Flower sampling: discussion

Flower thrips aggregate in flowers, attracted by flower colour and scent. An understanding of how the thrips select and move between flowers can help inform decisions as to the most appropriate flowers to sample for various purposes and aid in the interpretation of results. In short-lived flowers such as bindweed (*Calystegia sepium*), *Thrips major* move in and out of flowers within a single day (Kirk, 1985a). In field bean

(*Vicia faba*), both adults and larvae of *Kakothrips pisivorus* move along the raceme as new flowers open, resulting in an accumulation of larvae in flowers at the end of the raceme (Kirk, 1985b). In strawberry, adult *F. occidentalis* leave flowers as they senesce to colonise newly open flowers (Figure 2.3), probably in search of fresh pollen. Females require a richer diet than males for egg-production and move out of senescing flowers earlier than males. There were twice as many adults in top flowers than in side flowers. A number of factors may contribute to the selection of flowers by thrips: top flowers are usually more visible than side flowers because they stand proud of the foliage and because they have more sunlight illuminating them, also both males and females aggregate in prominent flowers for mating (Terry & Dyreson, 1996; Hamilton *et al.*, 2005). Selection of top flowers by *F. occidentalis* is also observed in other crops such as apple where more thrips are found in king buds than in lateral buds (Terry, 1991). On strawberry, eggs were observed in the sepals of green buds and hatching larvae were able to enter green buds and complete most of their development within a single strawberry flower under UK conditions. Numbers of larvae increased with flower age as more eggs hatched, although larvae may also move between flowers.

Larval numbers decreased sharply after flowering (Figure 2.3), which could be because they had completed their development and dropped to the ground to pupate (Holmes *et al.*, 2012), or because they moved into fresh flowers. Temperature may affect the population structure and abundance of *F. occidentalis* in flowers. Larval development time decreases with increased temperature, for example from 14.4 days at 16 °C to 11.2 days at 19 °C and to 5.5 days at 25 °C on strawberry (Nondillo *et al.*, 2008). As a result there is likely to be a greater proportion of larvae in flowers during the cool, cloudy conditions present during these experiments when flowers were open for 10–12 days, than might occur in warmer conditions or climates when thrips would pupate earlier and drop out of the flower. Bloom progression in the family Rosaceae is also temperature dependent (DeGrandi-Hoffman *et al.*, 1987) and a shorter flowering time at higher temperatures could also affect larval numbers.

The variation in *F. occidentalis* abundance and population structure between flowers has implications for sampling. The choice of flower selected by different samplers and in different growing systems (e.g. table top vs. ground produced strawberries) is likely to vary. In these experiments, flower selection could make a difference by as much as a factor of 4 for adult thrips (e.g. mature top flowers vs. senescent side flowers) or a factor of

3 for larvae (e.g. young flowers vs senescent flowers). The male: female ratio also changed between flower stages (e.g. from 1:3 in young flowers to 1: 1 in mature flowers on 5 July). Under the conditions of these experiments the sampling of adult *F. occidentalis* in mid-aged mature flowers (fully open flowers, with petals intact, and pollen starting to dehisce) was likely to result in the most accurate population estimates as sampling newly opened flowers could underestimate the numbers of males and sampling senescent flowers could underestimate the numbers of females.

Visual assessment of larvae is not recommended for population estimates as larvae are difficult to see in flowers and usually underestimated (González-Zamora & Garcia-Marí, 2003) and because numbers vary significantly between flower stages and temperatures. When an estimate of larval populations is required, for example to determine the efficacy of the larval predator *Neoseiulus cucumeris*, the sampling of senescent flowers may be most appropriate, where larvae are most abundant. The numbers of larvae increase with flower age as more larvae hatch out, then decline sharply after senescence (Figure 2.3). The sampling of young flowers underestimates numbers of larvae as some would not have hatched yet and the sampling of older fruit would exclude larvae that had pupated and those that had moved into fresh flowers to feed on pollen.

At the start of a flower flush or at the end of flowering it may not be possible to select a specific flower stage to monitor because it could be absent or scarce, resulting in a shift in thrips numbers and population structure that reflects the stage of flower sampled rather than a change in the population. For example, only young flowers might be available at the start of flowering resulting in a higher proportion of females and an underestimate of larvae. This needs to be taken into consideration when interpreting results. This work demonstrates the importance of being consistent and precise in flower selection when developing thresholds or estimating populations of *F. occidentalis* in strawberry, specifying the age and position of flowers and selecting the most appropriate thrips stage to sample according to the purpose of monitoring.

Comparison between counts by eye of adult thrips in mature mid-aged strawberry flowers carried out by different samplers, with counts of thrips in flowers from alcohol samples confirmed that the method of sampling was sufficiently accurate and could be carried out by farm staff following a minimum of training. Rapid assessment can result in a low estimate of thrips numbers (e.g. sampler 2, Table 2.3), so it is important to train and

test samplers on farm before relying on their counts for decision-making. Feedback from farm staff on the training sheet indicated that it could be improved by the addition of photographs of insect species that look similar to thrips and are frequent in strawberry flowers, such as springtails (symphilids).

This study found that counting the numbers of adult thrips in mature, mid-aged flowers by eye resulted in a reliable estimate of the thrips population, and that this method was practical for use by growers, so was used throughout the rest of the project. To ensure a consistent sample, flowers were always sampled from the top (rather than the side) of plants. While this would result in a higher population estimate, such flowers are most at risk from thrips damage, so provide growers with an earlier warning of potential fruit damage.

2.6. Environmental measurements

Temperature and humidity was recorded every 30 minutes during all the UK experiments in strawberry. A data logger (EL-USB-1, Lascar Electronics, Salisbury, UK) was suspended in the crop canopy at about flower height in a white delta trap (273 mm length, 130 mm height, œcos, Kimpton, UK) to shade it from the sun. The delta traps were placed centrally in each experimental plot with the base of the traps resting on the strawberry bed or growbag between the strawberry plants and secured with bamboo canes. Data loggers were suspended in the air within the traps on a wire looped through a hole in the trap and the data logger cap.

2.7. Rearing of *Frankliniella occidentalis*

A stock culture of *Frankliniella occidentalis* was maintained to provide a reliable source of one species of thrips for controlled damage experiments, as these could not be obtained from strawberry fields, where mixed-species thrips populations were prevalent. *Frankliniella* species can be reared on a variety of host plants including beans (*Phaseolus vulgaris*) (Murai & Loomans, 2001), cucumbers (*Cucumis sativus*) (DeGraaf & Wood, 2009), chrysanthemums (*Dendranthema grandiflora*) (van Dijken *et al.*, 1993), as well as leaf discs (Teulon, 1992) and artificial diets (Murai & Ishii, 1982). The presence or

addition of pollen increases the fecundity of *F. occidentalis* and therefore the productivity of a culture (Trichilo & Leigh, 1988).

In these studies *F. occidentalis* was reared on potted chrysanthemums, *D. grandiflora*, which was easy, low maintenance and productive. Thrips have been reared at Keele University since 1997, with the original thrips collected from UK glasshouses (Kirk & Hamilton, 2004). Plants of various cultivars and colours were sourced from supermarkets (Sainsbury's, Morrison's or Tesco, Newcastle under Lyme, UK). Rearing cages (height 600 mm, width 430 mm, depth 430 mm) (Figure 2.5) had transparent perspex sides (Rubberfast Ltd., Fenton, UK), open bases and clear plastic sheeting roofs to let light into the cages. Access was gained through a detachable front panel (width 370 mm, height 540 mm), held by two wing nuts. Cages were stood on capillary matting (Vattex Black, double-layer, Berrycroft Stores Ltd., UK) in a base tray (500 mm²). Each cage was ventilated by a fan (12 V, 0.8 W, Papst-Motoren, St. Georgen, Germany) at the back, with two air vents in the front panel to prevent condensation. There were six cages and four chrysanthemum plants per cage. The oldest plant in each cage was replaced with a new plant every 4-7 days according to thrips demand and plant quality. The culture was maintained at 25 ± 2°C, 60-80% RH and 16 h light: 8 h dark regime. Full-spectrum fluorescent tubes were used to provide a UV component similar to daylight (58 W Sylvania Activa 172 professional, Germany). Each tray was watered with about 500 ml tap water, three times a week, which was sufficient to maintain the plants without flooding the base tray and matting, so allowing any pupae there to survive and develop.

The success of *F. occidentalis* rearing can be affected by a number of factors, such as temperature and humidity, micro-climate, host-plant quality, and infestations of predatory mites (Loomans & Murai, 1997). Over the course of the three years of this study the rearing method was considered robust, easy to use and produced very large numbers of thrips. However, overwatering resulted in a higher humidity within the cages, which increased the incidence of fungal diseases such as powdery mildew (*Erysiphe cichoracearum*), and increased incidence of the predatory mites *Neoseiulus cucumeris* and *Macrocheles robustulus* and of the ground beetle, *Dalotia coriaria*. Predators are used increasingly by commercial growers to control *F. occidentalis* in glasshouse chrysanthemums and were observed on plants bought from the supermarkets. DeGraaf and Wood (2009) developed a method of rearing that reduced predatory mite infestation, although labour for maintaining the cultures was increased. Predatory mite egg hatch is

affected by low humidities, and I observed that reducing the humidity in the cages (by reducing the amount of watering) and replacing the plants more frequently was an alternative method of reducing the predatory mite population with minimal labour input. If this observation is demonstrated experimentally, then conversely growers might be able to improve predatory mite efficacy by increasing the relative humidity in glasshouse crops, although the effect on plant diseases would also have to be tested.

2.8. Statistical analysis

Statistical analysis was carried out using Minitab 16 (Minitab Incorporated, Pennsylvania, USA). Data and residuals were checked for normality using an Anderson-Darling test. In most cases parametric analysis of variance or regression was used on $\log_{10}(n+1)$ transformed data to homogenise the variance unless stated otherwise. Multiple comparisons used Tukey's test. Where data were not normally distributed, Kruskal-Wallis tests adjusted for ties were used. Multiple comparisons used repeated pair-wise Mann-Whitney tests. Data were considered statistically significant where $P < 0.05$. Where multiple comparisons are made, PEPI version 4.0 (Programs for EPIdemiologists) (Abramson & Gahlinger, 2001) was used to obtain Holm's adjusted P -values. This procedure was proposed by Holm (1979) and recommended by Wright (1992).

Tables and figures show untransformed means to aid interpretation and allow more intuitive comparison with counts that would be used by growers, whilst statistical analysis used transformed data. The percentage or ratio comparisons between treatments and the controls were calculated by comparing the untransformed means.

Table 2.1. Field sites and growing methods used for trapping and monitoring experiments in semi-protected strawberry crops in the West Midlands UK, 2011 to 2013.

| Field No. | Location (field name) Grid reference | Area (ha) | No. of tunnels (width, m) | Growing System | Cultivar | Crop year |
|-----------|---|-----------|------------------------------|----------------|-----------------------------------|----------------------------------|
| 1 | Tamworth (Block 4) N 52° 37' 23.54" W 1° 45' 31.83" | 2.0 | 22 (6.5 m) | Raised beds | Albion | 3 (2011) |
| 2 | Stafford (Toft) N 52° 46' 6.44" W 2° 10' 14.14" | 12.5 | 134 (8 m) | Raised beds | Camarillo | 2 (2011) |
| 3 | Tamworth (Taylor's) N 52° 37' 19.73" W 1° 45' 17.76" | 2.1 | 14 (6.5 m) | Growbags | Camarillo Camarillo Finesse | 2 (2011) 1 (2012) 1 (2013) |
| 4 | Tamworth (Road) N 52° 37' 43.89" W 1° 46' 10.88" | 2.4 | 22 (6.5 m) | Raised beds | Camarillo | 2 (2012) |
| 5 | Tamworth (Block 5) N 52° 37' 27.99" W 1° 45' 27.45" | 2.0 | 22 (6.5 m) | Raised beds | Finesse | 1 (2012) |
| 6 | Tamworth (Quarry) N 52° 37' 57.72" W 1° 46' 11.86" | 2.4 | 17 (6.5 m) | Raised beds | Finesse | 2 (2012) |
| 7 | Tamworth (12 acre) N 52° 37' 22.59" W 1° 45' 51.58" | 1.0 | 8 (6.5 m) | Raised beds | Finesse Finesse | 1 (2011) 2 (2012) |
| 8 | Tamworth (Meadow) N 52° 37' 16.28" W 1° 45' 24.30" | 1.0 | 8 (6.5 m) | Growbags | EME676 | 1 (2012) |
| 9 | Stafford (a) N 52° 43' 46.83" W 2° 16' 13.03" | 21.4 | 40 (8 m) | Raised beds | Camarillo | 1 (2012) |
| 10 | Stafford (b) N 52° 44' 05.83" W 2° 16' 24.63" | 21.4 | 40 (8 m) | Raised beds | Camarillo | 2 (2012) |
| 11 | Lichfield (Hanch 7) N 52° 43' 5.48" W 1° 51' 01.37" | 4.5 | 30 (6.5 m) | Raised beds | Camarillo | 2 (2013) |

Table 2.2. Description of the strawberry flower stages monitored for *F. occidentalis*

| Flower stage | Description of flower stage |
|------------------|--|
| Green bud | Sepals forming a ball enclosing the petals, no petals showing. |
| White bud | Sepals opening, petals showing but not open, no anthers visible. |
| Young flower | Sepals open, fresh petals, anthers opening, pollen visible, fresh pistils. |
| Mature flower | All petals present, not withered, pollen shed and anthers darkened. |
| Senescent flower | At least one petal present, petals withered, drying anthers and styles |
| Button fruit* | No petals, receptacle protruding from sepal whorl, green seeds visible. |

* The button fruit is the young green fruit, where the seeds cover a greater area of the fruit than the flesh between.

Table 2.3. The mean numbers of adult thrips \pm SEM per flower and comparisons between different samplers compared to counts from flowers in alcohol, from fields with moderate ($F_{(4, 145)} = 8.24$, $P < 0.001$) and low ($F_{(4, 145)} = 1.48$, $P = 0.21$) thrips numbers, in a UK strawberry crop ($n = 30$ flowers). Means followed by the same letter within each row are not significantly different ($P > 0.05$). The table shows untransformed means whereas the statistical analysis used log transformed data.

| Field no.* (cv.) | Mean numbers ± SEM of thrips per flower | | | | Alcohol samples |
|------------------------|---|------------------|------------------------|-----------------|--------------------|
| | Experienced samplers | | Inexperienced samplers | | |
| | 1 | 2 | 3 | 4 | |
| Field 7 (Finesse) | 7.0 ± 0.50 a | 3.2 ± 0.50 b | 6.0 ± 0.78 a | 6.6 ± 0.67 a | 5.7 ± 0.69 a |
| Field 3 (Camarillo) | 0.07 ± 0.06 a | 0.10 ± 0.05 a | 0 ± 0 a | 0 ± 0 a | 0.03 ± 0.03 a |

* Field number refers to Table 2.1



Figure 2.1. Examples of field sites of everbearer strawberry crops grown under semi-protected polytunnels showing (A) a second-year crop in July, grown in raised beds irrigated by T-tape (field 3 in Table 2.1) and (B) a first-year crop in April, grown in growbags with drip irrigation (field 2 in Table 2.1).

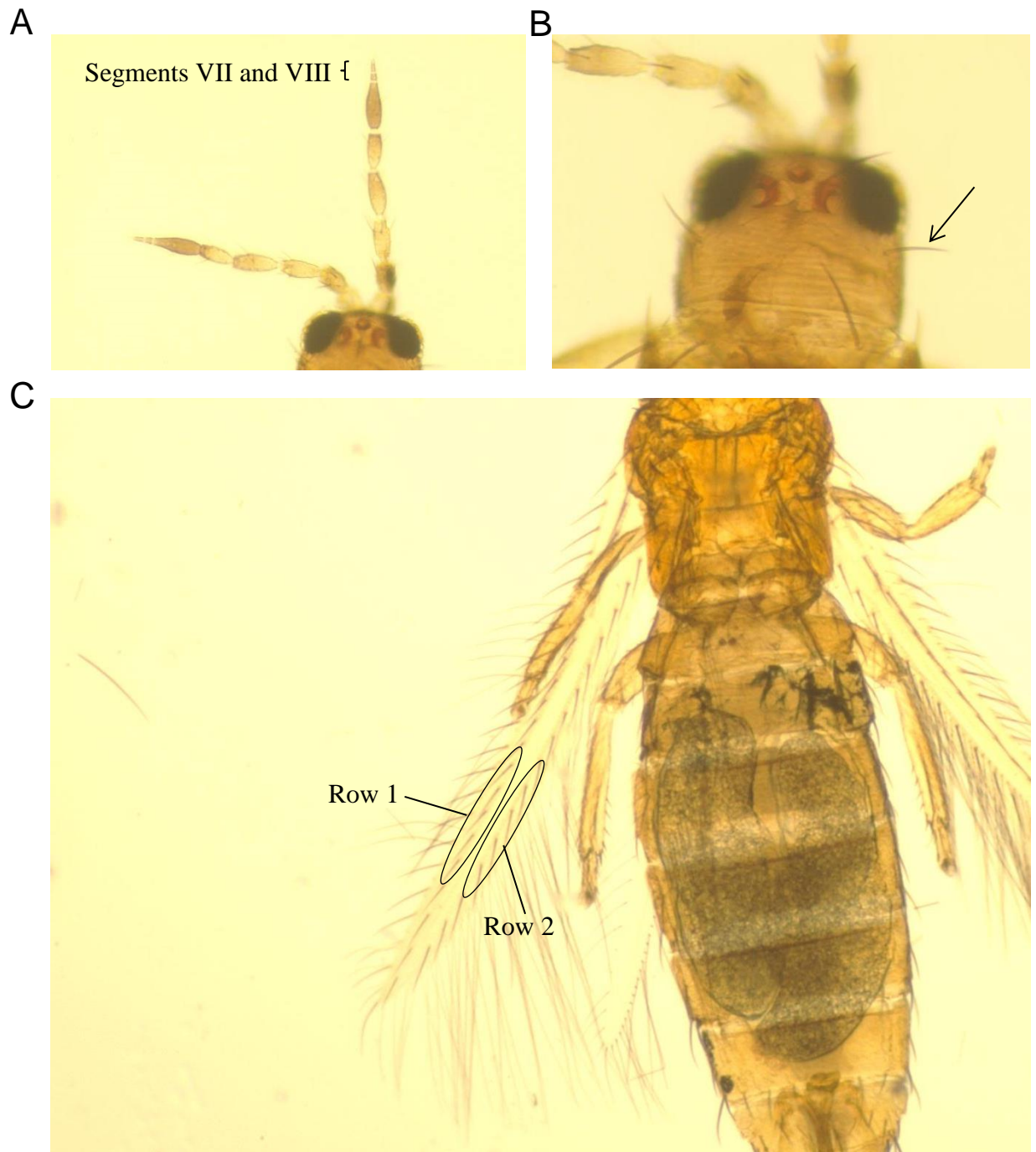


Figure 2.2. Main identification features of *F. occidentalis* showing (A) antennae with eight clear antennal segments (B) post-ocular setae about three times as long as other post-ocular setae and (C) the forewing with two complete rows of setae between the hair fringes.

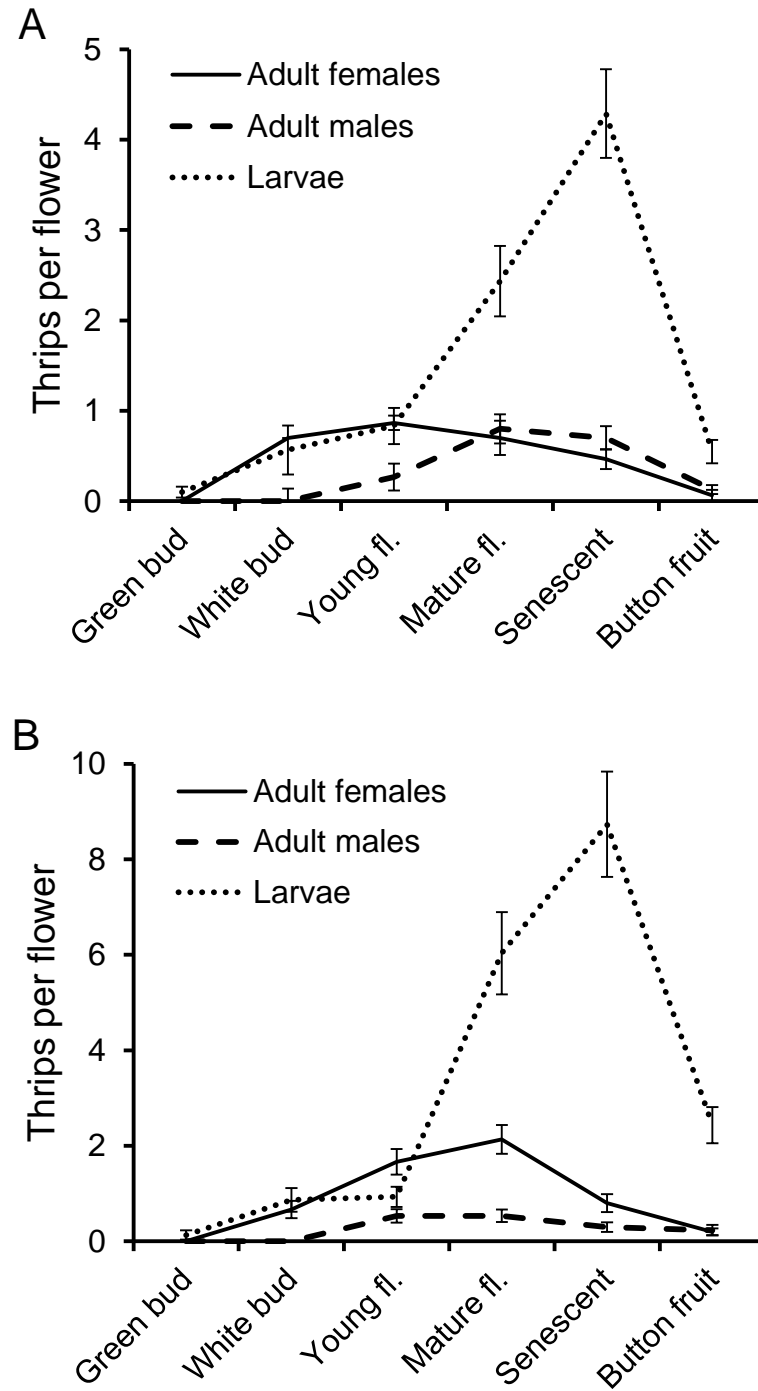


Figure 2.3. The mean number \pm SEM of thrips in different flower stages in a commercial UK strawberry crop (cv. Camarillo) on (A) 5 July 2011 and (B) 12 July 2011.

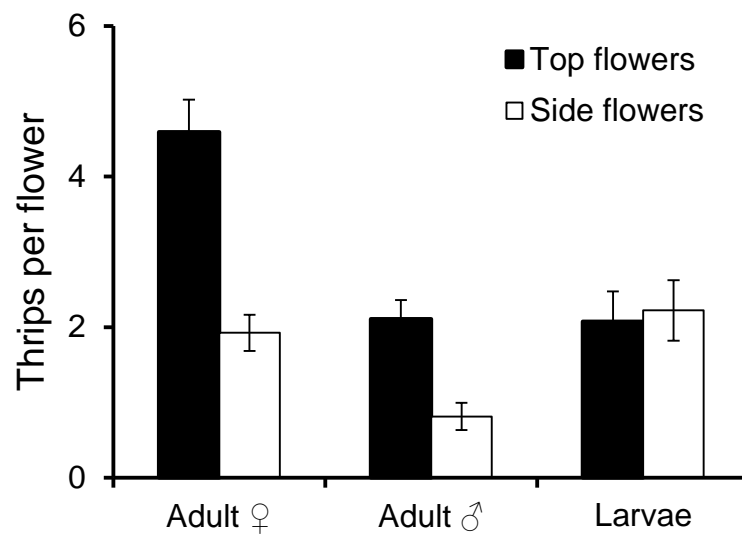


Figure 2.4. The mean number \pm SEM of thrips in top or side mature flowers in a commercial UK strawberry crop (cv. Camarillo).



Figure 2.5. Perspex cages, with fans and vented doors, used for rearing *Frankliniella occidentalis* on potted chrysanthemum (*Dendanthema grandiflora*) at $25 \pm 2^\circ\text{C}$ under full spectrum lighting (turned off for the photograph).

Chapter 3

Phenology in strawberry

3.1. Introduction

An understanding of factors that affect the severity and timing of *F. occidentalis* infestations is needed by growers to predict outbreaks, so that timely interventions and effective management programmes can be put into place. This chapter examines seasonal fluctuations and the distribution of *F. occidentalis* within and between semi-protected strawberry crops in the West Midlands area of the UK, in order to identify factors affecting population development, such as temperature, food availability, overwintering and natural enemies (Kirk, 1997b).

Temperature limits the range and abundance of cold-blooded animals like thrips, with upper and lower thresholds for development and survival (McDonald *et al.*, 1998). In a classic study, *Thrips imaginis* was sampled almost daily in rose flowers over a 14-year period in Australia, where seasonal fluctuations could be largely explained by temperature and rainfall data, with thrips populations increasing as soon as temperatures are suitable for development and crashing when it is too hot and dry (Andrewartha & Birch, 1954), although insufficient account may have been taken of density-dependent factors in their study (Smith, 1961). Temperature-based models can predict *F. occidentalis* population development reasonably well, early in the season, in some glasshouse cucumber and chrysanthemum crops (Wang & Shipp, 2001; Nothnagl *et al.*, 2008) and broad risk of an outbreak in outdoor crops (Olatinwo *et al.*, 2011), but are less good once thrips population growth is limited by competition for resources or by natural enemies.

In UK strawberry, early-season *F. occidentalis* population development may be restricted by low temperature. Minimum temperature for *F. occidentalis* development has been estimated at between 6.7 and 11.8°C, by assuming a linear relationship between development rate and temperature, and extrapolating the temperature down from data points tested at higher temperatures (mostly between 15 to 30°C) (Table 3.1). Because there is high mortality and possibly a non-linear relationship at the extremes, and the

threshold may differ for each life stage, the thresholds should be viewed with some caution (Bergant & Trdan, 2006). Some of the variability may result from different biotypes, cold-hardening or from local adaption to cooler climates (Felland *et al.*, 1995; McDonald *et al.*, 1997b; Brunner & Frey, 2010). Once above the threshold, development time decreases in a linear relationship with temperature between 10 and 35°C, with optimum temperatures of 25 to 29°C and high larval mortality above 35°C (Lublinkhof & Foster, 1977; van Rijn *et al.*, 1995; McDonald *et al.*, 1998). Egg-laying rate increases with temperature (Robb, 1989) and population growth did not occur below 15°C on cucumber (Gaum *et al.*, 1994). On strawberry, egg to adult development time is around 33 days at 16°C (typical mean early-season temperatures in UK polytunnels), 13 days at 25°C (typical mean summer temperatures in UK polytunnels) and 10 days at 31°C (Nondillo *et al.*, 2008). Reported temperature requirements to complete a generation (egg to adult) vary between 114 to 268 day-degrees above a minimum temperature (7 to 11°C) (Table 3.1), allowing for completion of three to five generations per year outside in the West Midlands area of the UK or 11 to 18 generations in Brazilian strawberry (Sites & Chambers, 1990; McDonald *et al.*, 1998; Nondillo *et al.*, 2008). The number of generations that occur in semi-protected UK strawberry is not known and is estimated in this study.

Frankliniella occidentalis is a predominantly a flower-inhabiting species, and the flowering pattern is likely to be a key factor affecting its population growth in strawberry. The presence of pollen increases fecundity and reduces development time in *F. occidentalis* (Trichilo & Leigh, 1988), almost doubling the intrinsic rate of increase on cucumber (Hulshof *et al.*, 2003). On strawberry, at 25°C, females produced nearly eight times more offspring (70 offspring per female) in the presence of flowers compared to leaves alone (Nondillo *et al.*, 2009). In chrysanthemum, there are conflicting data: Gerin *et al.* (1999) found limited *F. occidentalis* population growth without flowers, whilst Nothnagl *et al.* (2007) found little difference in population growth between flowering and non-flowering plants. As thrips population density increases, competition between thrips for space and resources within flowers reduces the oviposition rate (Kirk, 1994; O'Leary, 2005), which is likely to be a limiting factor in UK strawberry. Further information is required on the effect of the timing of flowering or flower density on *F. occidentalis* population development in strawberry.

Natural enemies limit the growth of most animal populations and control of pesticide-resistant *F. occidentalis* relies on inundative releases of predators in UK protected crops

(Jacobson, 1997) (see Chapter 1, Table 1.3). The use of natural enemies for thrips control was being investigated by other science partners within this project, so was beyond the scope of this study. However, limited sampling of important natural enemies, such as *Neoseiulus* spp. and anthocorids was carried out and the impact of *N. cucumeris* on thrips damage and economic injury levels is tested in Chapter 4.

The presence of flowering weeds within and surrounding crops could also affect the phenology of thrips in strawberry. *Frankliniella occidentalis* adults have been collected from well over 100 species of flowering weeds, e.g. (Chamberlin *et al.*, 1992; Chellemi *et al.*, 1994; Cho *et al.*, 1995a; Katayama, 2006). Thrips larvae were counted in these studies, but not usually identified to species because of a lack of suitable identification keys, however Kahn *et al.* (2005) reared larvae through to adults for identification and found that the distribution of *F. occidentalis* larvae between host plants matched that of the adults. In controlled trials, weed density had limited or no effect on seasonal abundance of *F. occidentalis* in apples or field beans (Cossentine *et al.*, 1999; Cockfield & Beers, 2008; Atakan, 2010), possibly because of the low abundance of weed flowers compared to a flowering crop. However, sudden influxes of thrips and associated damage have been observed when weed control results in movement of thrips onto a crop. Weeds also provide overwintering sites that could increase the carry-over of thrips between crops (Chamberlin *et al.*, 1992; Cho *et al.*, 1995a). Conversely, weeds may contribute to thrips control by providing food and refuge for key natural enemies, such as anthocorids and predatory mites (Atakan, 2010). Predators survive on pollen and nectar supplied by the weeds when they would otherwise decline or die out, and then move back into the crops at flowering (Van Rijn *et al.*, 2002). In such cases, the weeds are working as banker plants which have improved *Orius* spp. establishment in ornamental crops. Full investigation into the effect of weeds on thrips populations in strawberry was beyond the scope of this study, but initial studies were carried out to identify whether common weed hosts are present in UK strawberry crops and whether these contribute to the overwintering of *F. occidentalis*.

The ability of *F. occidentalis* to overwinter in semi-protected strawberry affects early-season abundance. *Frankliniella occidentalis* overwinters outside in southern USA and southern Europe, but not in the cooler climates of Canada and Denmark (Chambers & Sites, 1989; Broadbent & Hunt, 1991; Brødsgaard, 1993a). Most records of overwintering are of adults (Chambers & Sites, 1989; Chamberlin *et al.*, 1992), which are hardier than pupae and larvae (Brødsgaard, 1993a; McDonald *et al.*, 1997b). As the pupal stage is

short-lived, it could be that the pupae hatch as adults before the spring. Pupae are recorded through the winter in California (Campbell *et al.*, 2012), but this could be the result of warmer temperatures there. Typically, *F. occidentalis* (all stages) survives for less than a week at constant temperatures below 0°C, although adults overwintered in NE USA in the soil and in the open air after 35 consecutive nights below zero, suggesting that warmer daytime temperatures allow some recovery (Felland *et al.*, 1993, 1995; McDonald *et al.*, 1997b). In the UK, *F. occidentalis* has predominated in glasshouses where it can breed throughout the year when conditions are suitable, as there is no obligate diapause (Brødsgaard, 1994; Ishida *et al.*, 2003). Outside, it can survive in southern UK in mild winters and for short periods of cold weather, but prolonged chilling results in high mortality (90 to 100%) and an experiment showed no overwinter survival on leaf discs in the Midlands (McDonald *et al.*, 1997a-b; Bale & Walters, 2001). Exposure to cold temperatures also reduced the reproductive output of survivors by about half (McDonald *et al.*, 1997a), so winter temperatures could have a critical effect on both survival and early season population growth. Further data were required to confirm whether adults overwinter in strawberry crops in the West Midlands and to determine the impact of this on early-season thrips populations.

There is limited knowledge on thrips dispersal within and between fields. The Australian gall-forming thrips, *Oncothrips tepperi*, disperse 1 km between trees (McLeish *et al.*, 2003) and *Frankliniella tritici* migrates tens of kilometres annually to infest strawberry fields in north-eastern USA (Felland *et al.*, 1994). Although *F. occidentalis* may be carried on the wind, its flight in protected crops is typified by short flits rather than sustained flight. Spread from a central release point in glasshouse chrysanthemum and cucumbers was estimated as 0.2-0.3 m per day, at 21-27°C (Rhainds & Shipp, 2004). The movement of *F. occidentalis* around the world in recent years is thought to be through the plant trade rather than by flight (Kirk & Terry, 2003). Further information is required on the distribution and spread of *F. occidentalis* within and between UK strawberry fields to help predict which crops or areas within crops are most at risk from thrips damage.

Other factors could play a role that was not investigated here: Daylength affects bionomics with shorter larval development time and increased female survival at longer daylength (Brødsgaard, 1994; Whittaker & Kirk, 2004). The strawberry cultivar is known to affect egg hatch, female longevity and population growth (Rahman *et al.*, 2010). Relative humidity affects adult survival at the extremes, but high humidity can also

increase mortality by favouring fungal diseases such as *Beauveria bassiana* (Shipp & Gillespie, 1993; Shipp *et al.*, 2003). Cultural techniques that affect plant vigour and health also affect *F. occidentalis* population growth (Scott Brown *et al.*, 2002; Chau *et al.*, 2005).

The overall aim of this chapter was to identify the main factors affecting *F. occidentalis* population development in semi-protected strawberry crops in the West Midlands area of the UK. Specific aims were to:

- (1) collect baseline phenological data on thrips density, thrips species and flight periods in relation to temperature, humidity and flowering periods, to identify key factors affecting the distribution and abundance of thrips within strawberry fields;
- (2) determine which thrips species are present in strawberry through the season;
- (3) identify weed hosts that could affect the phenology of *F. occidentalis* in strawberry crops;
- (4) determine whether active stages of *F. occidentalis* overwinter on strawberry and on selected weed hosts;
- (5) test whether there is a difference in *F. occidentalis* distribution and abundance between first and second-year crops;
- (6) test whether thrips distribution within polytunnels can be explained partially by local temperature gradients.

3.2. Materials and Methods

For the rest of this chapter ‘thrips’ refers to species in the family Thripidae, unless stated otherwise. Counts of thrips in flowers in all experiments were carried out by eye using a $\times 7$ head lens (optiVISOR, LightCraft, London, UK), in medium-aged mature flowers (petals open, and with anthers starting to dehisce) (Sampson & Kirk, 2012). Eye counts were used because the results could then be related to grower monitoring which is done in the same way and was sufficiently accurate to compare changes in thrips abundance (see Chapters 2 and 4).

3.2.1. Seasonal abundance

Baseline phenological data were collected from four everbearer crops (two in 2011 and two in 2012) on thrips density, thrips species and flight periods in relation to temperature, humidity and flowering periods. Data on fruit numbers and fruit damage were collected at the same time, but this is reported in Chapter 4.

Seasonal abundance was monitored in two second-year crops in 2011, each on a separate farm that had a history of *F. occidentalis* damage (cv. Camarillo, fields 2 and 3, Table 2.1). The growers continued with their usual thrips control programmes, which included releases of predators and insecticide treatments (Table 3.2). Field 2 was monitored from 2 March 2011 (before flowering) to 13 September 2011 (when the crop was ploughed up). Field 3 was monitored from first flowering on 17 May 2011 (earlier flowers had been de-blossomed as is common commercial practice) to 18 October 2011 (when the crop was pulled out of the growbags).

Flight periods were measured using blue sticky pheromone traps. In each crop, a blue sticky monitoring pheromone trap (25 cm × 10 cm, Impact trap, Russell IPM, UK) was set up in each of two separate tunnels. Blue sticky pheromone traps were used as they are known to be most attractive to *F. occidentalis* (see Chapter 5). Traps were placed in separate tunnels, 20 m apart and 20 m in from the ends of the tunnels to reduce sunlight and edge effects. Traps were placed vertically (south facing, landscape orientation) onto metal posts (60 cm) with the bottom edge of the traps about 10 cm above the crop and secured using rubber bands (size 33, Censtretch, Rochester, UK). Pheromone lures (Thripline_{ams}, Syngenta Bioline Ltd, UK), each containing 30 µg of the *F. occidentalis* aggregation pheromone, neryl (*S*)-2-methylbutanoate, were placed in pheromone lure holders (55 mm long, 25 mm diam., Russell IPM Ltd) slotted over the metal posts using a small wire loop, with the cage hanging on the north side of the trap, shaded from direct sunlight. Traps and pheromone lures were replaced weekly to ensure that the traps did not become contaminated with dirt and other insect species and so that the pheromone release rate was similar between weeks. Collected traps were placed in separate polythene wrappers and stored in a freezer. The numbers of thripids on traps were counted under a binocular microscope in the laboratory, using methods detailed in Chapter 2.

Thrips numbers on the crop were assessed weekly on 10 plants within 10 m of each trap. Plants were selected at random. On each plant, the total numbers of flowers and fruit

per plant and the numbers of adult and larval thrips in one medium-aged flower were counted as above. Counts by eye of larval thrips in flowers should be interpreted with some caution as the yellow larvae are easily missed amongst the yellow stamens and the counts are not as reliable as counts of adults (González-Zamora & Garcia-Marí, 2003). However, they would give a record of relative abundance through the season. The presence or absence of the predators *Orius* spp. (adults or nymphs) per flower and *Neoseiulus* spp. (actives) per fruit was recorded. The predatory mites were not routinely identified, but 20 *Neoseiulus* spp. individuals were selected at random from the flower alcohol samples (see below), mounted on slides, using methods for mounting thrips described in Chapter 2, and identified to species under a compound microscope (Leica).

The flowers were pooled and placed in 70% alcohol so that thrips could be extracted and the species identified using the methods detailed in Chapter 2. These data were combined with thrips identifications from other experiments and are reported in section 3.2.2. A simple estimate of the numbers of thrips per plant for the purpose of comparison was made by multiplying the mean numbers of adult thrips per flower by the mean numbers of flowers per plant. Flower counts included all stages, from bud to senescence that had at least one petal. Although this is an underestimate, as it does not include thrips on the fruit, leaves or off the plant, it is a relative measure of the thrips population for looking at variation over time. In whole plant counts in different fields during the season, about 74% of the *F. occidentalis* adult population was found in flowers (see Chapter 4).

In 2012, more limited monitoring was carried out on a first- and a second-year crop (cv. Finesse, fields 5 and 7, Table 2.1) from fields that were close to each other on the same farm, and so had similar climatic conditions, growing methods and management. This allowed comparison of the thrips population development in a first- and a second-year crop. Whilst there was insufficient replication to draw conclusions about the effect of crop age on thrips populations, it would show broad differences that could be tested further. The grower continued with his usual thrips control programme (Table 3.2). Both fields were monitored from 22 March (first flowering) to 13 September 2011 (near the end of cropping). In both fields, ten plants were sampled weekly from near the top of each field (in equivalent positions) where thrips populations were known to be highest, but 20 m in from the field edges to reduce edge effects. The sampling was carried out as above, but without the blue sticky traps, predator, fruit or fruit damage assessments, as the interactions

between these were tested in separate experiments (Chapters 4 and 5). The presence or absence of thrips larvae was recorded, but no counts were made.

Temperature and humidity were recorded in the canopy of a central strawberry bed in a sample plot of each field, using methods detailed in Chapter 2.

The timing of the first occurrence of thrips adults and larvae was compared to mean temperature and flowering periods for all crops. A simple estimate of the rate of increase each week was calculated by dividing the numbers of adult thrips per plant by that of the previous week and this was compared to the timing of 100% flower occupancy.

The number of *F. occidentalis* generations during each crop (to the end of cropping) was estimated based on minimum development temperatures and thermal requirements for a generation calculated by McDonald *et al.* (1998) (7.9°C and 268 degree-days), and the mean values from published studies (9.0°C and 216 day-degrees) (Table 3.1). McDonald *et al.* (1998) data were selected because it was based on a UK *F. occidentalis* population and because they had a data point that was closer to the development threshold (10°C) than in most other studies (most did not test below 15°C), so was considered likely to be the best fit. The mean of all the studies in Table 3.1 was used as a comparison.

The relationship between thrips density on the crop and trapping efficiency through the season is shown in Chapter 6, and will not be repeated here.

3.2.2. Which thrips species are present?

At least eight species of thripids have been recorded from strawberry flowers in the UK (Easterbrook, 1991; Cross, 2003), but fruit damage is usually associated with *F. occidentalis*, so it was necessary to determine which species occurred through the season in this study, and the relative importance of *F. occidentalis*. Flower samples collected during the field monitoring in 2011 (section 3.2.1.), the field distribution surveys in 2012 (section 3.3.2.) and the mass trapping experiments in 2012 (Chapter 6) were pooled by site and date and rinsed in alcohol to remove the adult thrips. *Frankliniella occidentalis* was separated from other species by eye under a binocular microscope and the different species groups were counted. Sub-samples of thrips were then placed on microscope slides in polyvinyl lactophenol to confirm the identification under a compound microscope (see section 2.3). Confirmation of the identification (on slides) was carried out for 50 randomly selected specimens considered to be *Frankliniella* spp. and 50 randomly selected specimens

considered to be *Thrips* spp. per month from June to September for each field separately. If fewer than 50 specimens were available, then all were identified. In total, over 2000 thrips adults were identified to species.

3.2.3. Are there weed hosts within strawberry fields?

A survey was carried out to identify *F. occidentalis* weed hosts that could affect thrips abundance in strawberry. The survey was carried out on 28 October 2011 after the end of cropping, as weeds infested with *F. occidentalis* at this time could contribute to overwintering. A field with relatively few weeds (compared to other fields and farms in the study) was chosen for the survey (field 3, Table 2.1), as weeds present in this field were likely to be present in other fields. Weeds were collected from strawberry beds and from the area between polytunnels in a 20 m long \times 6.5 m wide length of polytunnel and were identified to species using Stace (2010). The sampled plot was randomly selected (using random numbers), and was at least 20 m in from the edge of the field to reduce the incidental occurrence of thrips from the hedgerows. Where present, flowers from the different weed species were collected and placed directly into 70% alcohol, so that thrips could be extracted and identified at a later date. If no flowers were present, then leaves were collected. As the flowers or leaves were different sizes, samples were taken that were approximately the same volume. This was measured by the amount that would fit easily into a 50 ml collecting tube, for example, about three dandelion flowers (*Taraxacum officinale*), five mayweed flowers (*Tripleurospermum inodorum*), 15 groundsel flowers (*Senecio vulgaris*) or >20 chickweed flowers (*Stellaria media*). The flowers or leaves were rinsed in alcohol to extract the thrips. *Frankliniella occidentalis* adults were separated from other thrips, identified to species and counted using the methods detailed in Chapter 2. Other thripid species and larvae were counted but not identified to species. No attempt was made to quantify the frequency of each weed species or numbers of flowers per plant. Whilst no conclusions could be drawn about the impact of the weeds on thrips populations, the survey was sufficient to identify potential weed hosts that could be evaluated further.

To test whether weed hosts were widespread in other fields, a broader survey was carried out. Three weed species (*S. media*, *S. vulgaris*, *T. officinale*) were selected from the initial survey that hosted *F. occidentalis* adults and thrips larvae, were common, widespread and flowered throughout the year (Table 3.4). Thus if widespread, they could have an impact on *F. occidentalis* phenology in strawberry. On 28 October 2011, the

presence or absence of the three target weed species was recorded in every second tunnel in the four study crops used in 2011 (fields 1, 2, 3, 7, Table 2.1). To confirm the presence of *F. occidentalis* on the selected weed species, three plants of each weed species were collected from an area known to be infested with *F. occidentalis*, in each study field. The flowers were removed and placed in 70% alcohol, and the presence or absence of *F. occidentalis* adults on each weed species was recorded per field using the extraction and identification methods detailed in Chapter 2.

Selected weed species were monitored through the winter to determine whether they contributed to the overwintering of *F. occidentalis* in strawberry fields (section 3.2.4.).

3.2.4. Do active stages of *F. occidentalis* overwinter?

Overwintering of *F. occidentalis* in strawberry crops would affect thrips abundance in the spring and the carry-over of thrips from first- to second-year crops could result in higher thrips numbers in second-year crops.

To determine whether active stages of *F. occidentalis* overwinter in West Midlands strawberry fields, strawberry flowers and selected weed species were sampled from 29 October 2011 (after cropping) to 22 March 2012 (before first flowering), in three strawberry fields that were infested with *F. occidentalis* in 2011 (fields 3, 4 and 7, Table 2.1). Three weed hosts were sampled that were widespread in strawberry fields and flowered through the year (*S. media*, *S. vulgaris* and *T. officinale*, see section 3.3.3), thereby providing a potential over-winter food source. The fields were sampled on 29 October 2011, 29 November 2011, 13 December 2011, 25 January 2012, 22 February 2012 and 22 March 2012.

On each sample date and in each field, plant material was sampled from the same area of crop (approximately 7 m × 10 m), at least 20 m in from the edge of each field to reduce edge effects. Five strawberry flowers, three *T. officinale* flowers, 10 *S. vulgaris* flowers and 10 *S. media* flowers were collected per field, on each sample date. Flowers were sampled, as they are a known overwintering site (Chamberlin *et al.*, 1992). Where no open flowers were present, senescent flowers or dead flowers were sampled. If no flowers were present, then an equivalent volume of leaves were collected. The flowers or leaves were placed directly into a tube containing 70% alcohol for storage. Thrips were extracted from flower samples by washing them in 70% alcohol, then *F. occidentalis* were separated from other thripid species, sexed, counted and the identification confirmed using the methods

detailed in Chapter 2. Thrips larvae were extracted and counted, but not identified to species. Adults rather than pupae were sampled as they gave a more immediate measure of potential increase and can lay eggs as soon as temperatures are suitable.

Blue sticky traps (with and without pheromone) were used to determine whether *F. occidentalis* was active (flying) during the winter. Two pairs of blue sticky traps (10 cm high by 25 cm wide, Impact trap, Russell IPM) were set up in each of the three fields above on 29 October 2011, using the methods detailed in 3.2.1. Traps were placed at least 20 m in from the ends of the crops to reduce edge effects with at least 20 m between pairs and 10 m between traps within a pair. The trap positions (with and without pheromones) were alternated within each pair, with the first position chosen randomly, using a coin toss, on each monitoring date. Each pair was placed in a separate tunnel and consisted of one trap with a pheromone lure and one trap with a blank lure. Pheromone lures (Thripline_{ams}, Syngenta Bioline Ltd), each containing 30 µg of the *F. occidentalis* aggregation pheromone, neryl (*S*)-2-methylbutanoate, were placed in pheromone lure holders (55 mm long, 25 mm diam., Russell IPM Ltd) slotted over the metal posts using a small wire loop, with the cage hanging on the north side of the trap, shaded from direct sunlight. The control traps used a cage containing a blank natural rubber septum identical to that used for the pheromone (6.3 mm diam. × 10.8 mm long; International Pheromone Systems Ltd, UK). All traps were south-facing with the printed grid side of the trap facing north. Traps and lures were replaced regularly (on 29 November 2011, 13 December 2011, 25 December 2011, 22 February 2012 and 22 March 2012) and the traps placed in a polythene wrapper and stored in a freezer at -20°C. All thrips on traps were examined under a binocular microscope in the Keele laboratory and the numbers of male and female *F. occidentalis* were counted using the methods detailed in Chapter 2. Other thrips species were not identified to species. Mean trap catches of *F. occidentalis* on traps with and without pheromone were compared using analysis of variance from traps collected in on 29 November and 22 March 2012. Two traps had dropped to the ground, so affected blocks were excluded from the analysis. Counts from traps in place from 29 November 2011 to 22 February 2012 were excluded from the analysis because of the very low numbers of thrips caught during this time (a total of 6 *F. occidentalis* on 36 traps).

Temperature and humidity were recorded at the approximate height of senescent strawberry flowers in the crop, which was 5 cm above the bed, using methods detailed in Chapter 2.

3.2.5. Between-field distribution and abundance: is there a difference in thrips distribution and abundance between first and second-year crops?

To test whether there was a difference in distribution and abundance of thrips in first or second-year crops, strawberry flowers were sampled approximately monthly, from mid-April (approximately two weeks after first flowering) to mid-August 2012, in three matched pairs of first- and second-year crops. Each pair was of the same cultivar and on the same farm, so that cultural methods were similar: Pair 1, fields 3 and 4 (cv. Camarillo); Pair 2, fields 5 and 7 (cv. Finesse); Pair 3, fields 9 and 10 (cv. Camarillo) (Table 2.1). The length and breadth of each crop was divided into ten equal divisions then sampled in a zig-zag pattern so that there were 10 sample plots, distributed systematically over each crop (Figure 3.6). On each sample date (monthly from mid-April to mid-August), ten medium-aged flowers were selected arbitrarily per plot and the number of adult thrips per flower was counted by eye (as above) ($n = 100$ flowers per field). In addition, ten fully swollen white fruit were selected from the same plants as the flower samples and the numbers of seeds surrounded by bronzing were counted by eye using a $\times 7$ head lens (optiVISOR) ($n = 100$ fruit per field). The fruit and flower data were used for determining damage thresholds (see Chapter 4). The flower samples were pooled and placed in 70% alcohol so that thrips could be extracted and the species identified using the methods detailed in Chapter 2. Mean thrips per flower in first- and second-year crops were compared per month and the percentages of flowers infested with thrips in the different crops were compared through the season. Analysis (ANOVA) used the mean thrips density per crop to avoid pseudo-replication ($n = 100$ flowers per crop).

The flower samples from the Camarillo crops were also used to determine the sample size required for estimating thrips populations (see Chapter 4).

3.2.6. Within-field distribution and abundance: do temperature gradients within fields affect local abundance?

As strong gradients in thrips density were apparent within strawberry fields, a survey of thrips distribution was carried out in a second-year crop to see whether the pattern of infestation could be explained. The crop was sampled systematically on 6 June 2012 (cv. Finesse, field 7, Table 2.1). Samples were taken from five plots in each of four different tunnels (alternate tunnels from a series of eight tunnels). In all fields the tunnels ran up the slope to aid irrigation. The first plot was 15 m in from the top of the field, then at every 40

m down the tunnel, so that the last plot was 15 m in from the bottom of each tunnel. At each sample point, five medium-aged flowers (as above) were selected arbitrarily per plot and the number of adult thrips was counted by eye per flower. Temperatures were recorded 15 m in from the top of the field and 15 m in from the bottom of the field during the time of sampling (10.00 am to 15.00 pm) using methods described in Chapter 2. Thrips abundance was compared between plots using ANOVA, at different distances down the tunnels and between tunnels.

As thrips were more abundant in the mid to top areas of the polytunnels sampled, where day-time temperatures are higher, this relationship was investigated further. To test the relationship between thrips density and daytime temperatures, the steepest sloping field on a farm was selected, where the thrips density was known to be variable and the gradient up the slope was 1 in 12 (4.8°), to exaggerate any differences in temperature (cv. Camarillo, field 4, Table 2.1). Four tunnels were sampled on 13 September 2012, between 11.00 h and 14.00 h, when temperatures were likely to be at their warmest. Four tunnels with a similar slope were selected, to allow comparison between tunnels at specific altitudes. The tunnels were about 158 m long with two tunnels (16 m) between each tunnel and two tunnels in from the edge of the field to reduce the influence of adjacent hedgerows and cooler wind currents coming from outside the crop.

Each polytunnel was sampled at eight points. The first plot was 2 m in from the top of the field, then at every 22 m down the tunnel, so that the last plot was 2 m in from the bottom of the field. At each position, the middle bed on the left of the tunnel was sampled, to provide a consistent sample in case temperatures varied across each tunnel. At each sampling position (36 plots) the following measurements were taken:

- The altitude was recorded in metres using a satellite navigation device (Nüvi, Garmin (Europe) Ltd., Southampton, UK).
- The temperature was recorded using a digital thermometer (Thermo-Hydro, RS 212-124, Oregon Scientific, Northants, UK) that was placed in a white delta trap (æcos, Hitchin, UK) to shade it from direct sunlight. The delta trap was placed on the strawberry bed, amongst the strawberry leaves and the temperature recorded after about five minutes, once the temperature had stabilised within the delta trap.
- The number of adult thrips per flower was counted by eye in the five medium-aged flowers (as above) that were closest to the thermometer position.

To inform possible temperature differences over a longer period of time, three data loggers recorded temperatures at 30 minute intervals from 24 September to 5 October 2012. The data loggers were suspended at flower height in delta traps (see section 2.6) about 20 m in from the bottom (95 m altitude), in the middle (101 m altitude) and about 20 m in from the top of a tunnel (107 m altitude).

The sampled flowers were pooled and placed in 70% alcohol so that the thrips could be extracted and the proportion of *F. occidentalis* determined, using the methods detailed in Chapter 2.

The difference in thrips density in flowers was compared between tunnels and at different heights up the tunnels using ANOVA. To test whether there was a correlation between thrips density and temperature, the mean numbers of adult thrips per flower were regressed on temperatures recorded at the different sampling points up the tunnels at the time of sampling.

3.2.7. Movement of adults between flowers

To help interpret the phenology results, a short observation was made to test the extent to which adult thrips move between strawberry flowers in the field. On 13 September 2012, ten medium-aged flowers were marked in a commercial semi-protected strawberry crop (field 4, Table 2.1). The flowers were selected at random within a 1 m × 4 m plot and were tagged with pegs placed at the base of the stem, taking care not to disturb the flowers or thrips within them. At hourly intervals, between 10.00 h and 13.00 h, each flower was examined carefully for about 30 seconds and the number of adult thrips per flower was counted by eye, using a ×7 head lens (optiVISOR). Unlike previous assessments, where petals were moved so that the thrips could be seen easily, the flowers were not touched, so that the thrips were not disturbed. Whilst some thrips may have been missed using this method, most would have been seen as the thrips were active at the time of the study and extra time was taken to observe each flower from all angles. Temperature was recorded using methods detailed in Chapter 2. The mean number of thrips between flowers and between times was compared. A simple measure of the movement was defined as whether or not the number of thrips per flower changed in an hour. Whilst this measure would underestimate movement, as it would not detect occasions when the same number of thrips move in and out of a flower, it was sufficient to give a broad indication of movement between flowers.

3.2.8. Statistical analysis

Statistical analysis is described in Chapter 2 (section 2.8).

3.3. Results

3.3.1. Seasonal abundance

Adult thrips, including *F. occidentalis*, were found in flowers within a week of first opening (late-March to mid-April) in all the second-year crops sampled (Figures 3.1 A, 3.2 A and 3.3 A). Larvae were found in the flowers three weeks after the first adults in an early-flowering crop, when temperatures averaged 11.5°C (Figure 3.3 A, D) and two weeks after the first adults in a late-flowering crop, when temperatures averaged 15.5°C (Figure 3.2 A, E). Later in the season, once the generations were over-lapping, larvae were observed in flowers as soon as they opened. In one isolated crop (there were no adjacent strawberry fields), there was a four-week delay between first adults (19 April 2011) and first larvae (17 May 2011), but there were so few thrips present in the crop that the larvae may have been missed considering the small sample size ($n = 20$ flowers) (Figure 3.1 A). In a first-year crop, no thrips were recorded until 23 May 2012, several weeks after the start of flowering on 22 March 2012 (Figure 3.3 A). This delayed infestation was observed in other first-year crops and was investigated further (see section 3.3.5). Thrips numbers increased steadily from flowering, then rapidly around mid-July and remained high for the rest of the season until late-September to early-October, when they declined again (Figures 3.1. C, 3.2. C, 3.3. C).

Blue sticky pheromone monitoring traps caught large numbers of thrips, averaging over 100 (field 2) and 800 (field 3) thrips per card trap per week (mid-May to mid-September) but exceeding 800 (field 2) and 2000 (field 3) thrips per monitoring trap per week on occasions. Linear regression analysis showed a significant correlation between weekly trap catch and mean adult thrips per plant (regression analysis, field 2, $F_{(1,40)} = 18.9$, $P < 0.001$, $R^2 = 30.4\%$; field 3, $F_{(1,38)} = 195.3$, $P < 0.001$, $R^2 = 83.3\%$).

In the two fields sampled in 2011 (cv. Camarillo), *Neoseiulus* spp. were recorded on 4% of fruit in field 2, where two releases of *N. cucumeris* were made (100 predators per m^2), and on 42% of fruit in field 3, where five releases of *N. cucumeris* were made (>1000

predators per m²) (Table 3.2). 95% of the *Neoseiulus* spp. collected from flowers were identified as *N. cucumeris* and 5% as *N. californicus* (n = 20). The efficacy of predatory mites was not tested in this study, but it was observed that the thrips population peaked at 10 adult thrips per flower in the field with the most predatory mites (field 3) and at 16 adult thrips per flower in the field with fewer predatory mites (field 2), even though field 2 started with fewer thrips (Figures 3.1 A, 3.2 A). *Orius* spp. were present in <1% of flowers in both crops. Naturally occurring *Anthocoris nemorum* was observed in flowers in very low numbers, late in the season. No other predators were observed in the flowers, although a few predatory thrips, staphylinid beetles and lacewings were observed on traps, which were not identified to species.

Thrips numbers per flower peaked at 20.6 in field 5 following a first flower flush with about 30 flowers per plant (compared to less than 10 in other crops) (Figure 3.3. A). There was insufficient replication to draw conclusions from this, as the crops were of different varieties and in different years.

Two trends were observed that could help to predict the timing of thrips damage: Increases in number of thrips per flower were observed at the end of flower flushes in second-year crops, as the thrips concentrated into fewer flowers (e.g. Figure 3.3. A, B); thrips density in flowers increased rapidly once thrips occupancy of flowers approached 100%, as before this any increase in thrips numbers results in a wider distribution between flowers (see section 3.3.7). These increases in numbers of thrips per flower did not necessarily coincide with an increase in the thrips population (thrips per plant). These trends were observed consistently between crops and years throughout the study, but further controlled experiments would be needed to confirm them.

Comparison of temperature records in the fields (from January to the end of cropping) with published data on the threshold for development and the number of degree-days required to complete a generation (using a UK data set and the mean of published data) suggested that five *F. occidentalis* generations could be completed by the end of cropping in semi-protected strawberry in the West Midlands (Table 3.3). The maximum increase in the adult population occurred when thrips approached 100% occupation of flowers (Table 3.3).

Relative humidity averaged 78.8 ± 2.4 % (range 55 to 95% RH) in the fields sampled, between mid-May and mid-September, whilst polytunnel covers were in place.

3.3.2. Which thrips species are present?

The thrips population in semi-protected strawberry was predominantly *F. occidentalis* in all the fields sampled at times when thrips numbers were highest and fruit damage was observed (typically July to September) (see Chapter 4 for damage records) (Figure 3.4). The proportion of *F. occidentalis* in strawberry flowers increased through the season to over 95% by the end of September. The proportion of *Thrips major* was relatively high early in the season (25 to 75% in June), but declined after June (Figure 3.4). Other thripid species present in low numbers included; *Thrips tabaci*, *Thrips fuscipennis*, *Frankliniella intonsa*, *Thrips atratus*, *Thrips angusticeps* and *Frankliniella tenuicornis*. All of these are common species known to breed on strawberry, except *F. tenuicornis*, which breeds on grasses and cereals. The predatory thrips *Aeolothrips intermedius* was also present in low numbers.

There was insufficient replication between crop age, crop variety, farm and insecticide use to draw conclusions on the impact of these on the abundance of *F. occidentalis*, but some general observations can be made. The thrips species and proportion of each through the season were similar in field 3 between 2011 (Figure 3.4. A) and 2012 (Figure 3.4. B), where a second-year crop was removed from growbags in October 2011 and new plants planted into the same growbags in February 2012. Fields 1, 2 and 3 were adjacent to other strawberry growing fields and had proportionally more *F. occidentalis* early in the season (52 to 63% in June) (Figure 3.4. A, C, F) compared to fields 9 and 10, which were more isolated fields with proportionally fewer *F. occidentalis* early in the season (0 to 25% in June) (Figure 3.4. D, E). There was a trend towards an increased proportion of *F. occidentalis* with increased pesticide-use. For example, in July, the proportion of *F. occidentalis* was 10% in a field where no spinosad (Tracer) sprays had been applied (Figure 3.4. E), 33%, 52% and 84% where one spinosad (Tracer) spray had been applied (Figure 3.4. F, A, B) and 99% and 100% where two spinosad (Tracer) sprays had been applied (Figure 3.4. C, F). Further work is required to test whether the increase in the proportion of *F. occidentalis* observed with pesticide use was the result of selective survival of pesticide-resistant strains.

3.3.3. Are there weed hosts within strawberry fields?

Frankliniella occidentalis adults were present on 15 different weed species from 10 different plant families in a semi-protected strawberry crop (Table 3.4). Adults were most

abundant on dandelion (*T. officinale*), scentless mayweed (*Tripleurospermum inodorum*) and black nightshade (*Solanum nigrum*). However, as *T. inodorum* and *S. nigrum* were only represented by a few plants in the sample area (<5 plants), and have limited flowering periods, they were not considered to be the most important weed hosts present. Thrips larvae were most numerous on weeds in the Asteraceae family. Although the larvae were not identified to species, it is likely that the majority were *F. occidentalis* in common with >95% of the adults at the time (September 2011, Figure 3.4 A) and because *F. occidentalis* has a wide host range and plants in the Asteraceae family are known hosts (Kahn *et al.*, 2005). Three weed species were selected as potentially important weed hosts on the basis that they were widespread, flower throughout the year and known hosts of *F. occidentalis* (adults and larvae). These were *Stellaria media*, *Senecio vulgaris* and *T. officinale* (Table 3.4).

In a wider survey, *S. media*, *S. vulgaris* and *T. officinale* were found to be widespread in strawberry beds, growbags and between the polytunnels, and were present in every strawberry tunnel of every field sampled (Figure 3.5). *Frankliniella occidentalis* adults and thripid larvae were extracted from all three weed species in all fields sampled. The survey confirms that there were many weed hosts in the fields sampled and if this is more widely the case, then weeds could affect the phenology in strawberry.

Although no attempt was made to quantify the effect of weeds on *F. occidentalis* phenology in strawberry, it was observed that thrips were sometimes more numerous beside weedy field margins (Figure 3.5 B) early in the season, and beside weedy areas between polytunnels (Figure 3.5 C) mid-season. Further studies are required to confirm these observations and to test whether weeds could be a refuge for important natural enemies.

3.3.4. Do active stages of *F. occidentalis* overwinter?

Frankliniella occidentalis overwintered as adult females in senescent or dead strawberry flowers and in the flowers of three common weed species found within the cropping area (*S. media*, *S. vulgaris* and *T. officinale*) (Table 3.5). Male *F. occidentalis* and thripid larvae were present in flowers on 29 November and 13 December 2011, but were not present in January, February or March 2012 (Table 3.5). This suggests that adult females are the only active stage that over-winters, although a more comprehensive survey in different micro-climates is required to confirm this. Adults could also be overwintering

in other areas of the crop, on other weeds species, in soil and plant debris or under the black plastic mulch, but these were not sampled. Pupae may be present in the soil or in the leaf litter around the base of strawberry plants, but these were not sampled.

Low numbers of *F. occidentalis* adults were caught on traps throughout the winter. The trap catches confirmed that it is predominantly females that overwinter, as males and females were caught in November and December 2011, but only females were caught from January to March 2012 before the polytunnels were erected. Blue sticky pheromone traps caught nearly three times more *F. occidentalis* than control traps in November and March, but the differences were not significantly different (one-factor ANOVA, $F_{(1, 21)} = 2.5$, $P = 0.13$), probably because of the low sample size (Table 3.5). Very few thrips were caught between December and late February, when mean temperatures were 2.5 to 5.5 °C and maximum temperatures were 11 to 12.5°C. Temperatures fell below 0°C on 29 nights, but not more than six consecutive nights and day-time temperatures always exceeded 0°C.

3.3.5. Between-field distribution and abundance: is there a difference in thrips distribution and abundance between first and second-year crops?

Thrips numbers were higher in second-year crops than in first-year crops, but the difference between them reduced as the season progressed, being significant from April to June, but not in July and August (Figure 3.7 A). No thrips were found in any of the first-year crops in April, but in all second-year crops (one-factor ANOVA, $F_{(1, 4)} = 8.4$, $P = 0.044$). Second-year crops had $\times 39$ more thrips in May (one-factor ANOVA, $F_{(1, 4)} = 17.7$, $P = 0.01$), $\times 24$ more thrips in June (one-factor ANOVA, $F_{(1, 4)} = 15.5$, $P = 0.017$), $\times 7$ more thrips in July (one-factor ANOVA, $F_{(1, 4)} = 2.6$, $P = 0.18$) and $\times 2$ more thrips in August (one-factor ANOVA, $F_{(1, 4)} = 1.3$, $P = 0.32$), than first-year crops.

The percentage flower occupation gives further insight into the spread of thrips within and between crops. The percentage of flowers occupied by thrips remained low (up to 15% flower occupation) in all the first-year crops from April to June. By comparison, there was 75-99% flower occupation in second-year crops by June. In July, whilst there was a small increase in thrips numbers per flower in first-year crops, there was a massive increase in flower occupation (Figure 3.7 B). This increase could have come from thrips within the crops, or from thrips invading from near-by crops and weeds, or a combination of both. Temperatures within the polytunnels were optimum for thrips flight during July (Figures 3.1 E, 3.2 E, 3.3 D) and second-year crops were usually in close proximity to the

first-year crops (e.g. within 500 m) and equipment such as picking trays was moved between fields regularly, so movement of thrips between crops on a farm is likely. Thrips numbers remained low throughout the season in a first-year crop that was isolated, newly planted in ploughed grassland and where predatory mites were released from planting (field 9, Table 2.1, Figure 3.7 B). In a second-year crop with a low proportion of *F. occidentalis* compared to *Thrips* spp. (Figure 3.4 D), there was a reduction in thrips flower occupation (from 79% to 35%) between June and July following a spinosad treatment (field 9, Table 2.1, Figure 3.7 B). Thrips were distributed throughout the fields from the start of the season in second-year crops. In first-year crops, in May, there appeared to be a bias towards more thrips around the edges of the fields, particularly beside weedy field margins, but this was not tested. By June this was no longer apparent.

3.3.6. Within-field distribution and abundance: do temperature gradients within fields affect local abundance?

In a survey of a second-year strawberry crop (field 7, Table 2.1), thrips were present throughout the field in early June (81% flower occupancy, 83% *F. occidentalis*), with the exception of one area at the bottom of the field that was cool and shaded by trees. The distribution of thrips was similar between the four tunnels sampled (two-factor ANOVA, $F_{(3, 19)} = 0.1$, $P = 1.0$), but within each tunnel there were $\times 17$ more thrips at the mid to top of the field compared with at the bottom end (two-factor ANOVA, $F_{(4, 19)} = 15.7$, $P < 0.001$) (Figure 3.8). Temperatures averaged about 1°C higher (17.3°C) near the top compared to the bottom (16.1°C) of the field at the time of sampling (10.00 am to 15.00 pm).

The relationship between temperature and thrips density within a polytunnel was examined further in a second-year crop in September (field 4, Table 2.1). There were $\times 6$ more thrips near the top of the field compared to near the bottom end (two-factor ANOVA, $F_{(7, 21)} = 13.8$, $P < 0.001$), but numbers dipped at the exposed tunnel ends (Figure 3.9 A, C). There was no difference in thrips numbers and distribution between the four tunnels sampled (two-factor ANOVA, $F_{(3, 21)} = 0.1$, $P = 0.94$). Temperature increased with altitude up the tunnels (Figure 3.9 B). When thrips numbers were regressed on temperature at different altitudes up the tunnels at the time of sampling there was a significant correlation between mean thrips density and temperature (regression analysis, $F_{(2, 5)} = 30.7$, $P = 0.002$; $R^2 = 89.5\%$).

The daily maximum and minimum temperature from top, middle and bottom positions up the tunnel from 24 September to 5 October are shown in Figure 3.10. Over the 13 day period, the mean maximum temperatures were about 4 to 5.5°C higher near the middle (25.7°C, 101 m) and top (27.5°C, 107 m) of a tunnel than near the bottom of the tunnel (21.9°C, 95 m) (one-factor ANOVA, $F_{(2, 36)} = 13.1$, $P < 0.001$). Temperature gradients were reversed at night, with a mean difference in temperatures of about 2°C between the top and bottom of the tunnel (Figure 3.10).

3.3.7. Movement of adults between flowers

The number of adult thrips in individual flowers was different from the number counted in the same flower one hour previously on 24 out of 30 occasions, indicating that adult thrips moved frequently between strawberry flowers (Table 3.6). Mean numbers of thrips per flower did not change between sampling times (two-factor ANOVA, $F_{(3, 36)} = 0.7$, $P = 0.6$), indicating that adults are moving between flowers rather than moving in and out of flowers. Adult thrips numbers were consistently higher in some flowers than others (two-factor ANOVA, $F_{(9, 30)} = 11.6$, $P < 0.001$) (Table 3.6), which is consistent with earlier studies showing that more thrips were found in flowers at the top of strawberry plants than at the side (see Chapter 2).

3.4. Discussion

This study has added to our knowledge of the distribution and abundance of *F. occidentalis* within and between semi-protected strawberry crops in the West Midlands area of the UK.

Frankliniella occidentalis is now the dominant thrips species on semi-protected strawberry on the farms sampled in the West Midlands, UK (Figure 3.4), as has been reported in other strawberry growing regions in the south of England, such as Kent and East Anglia (Cross, 2003). Before *F. occidentalis* arrived in the UK, *T. major* and *T. atratus* were the dominant thrips species on UK strawberry (Easterbrook, 1991), so *F. occidentalis* has displaced native thrips species in these crops as found in other protected crops in the UK (Jacobson, 1997) and in outdoor crops in warmer climates (Tung & Vierbergen, 1999).

Thrips major is one of the commonest flower-inhabiting thrips species in the UK (Kirk, 1996) and was present in similar numbers to *F. occidentalis* at the start of the season, but was gradually displaced as the season progressed (Figure 3.4). This contrasts with the seasonal abundance observed in non-cropped areas. In flowers in chalk grassland in Sussex, *T. major* is typically most abundant from July to September (Ward, 1973), so is a late-season species like *F. occidentalis* and both species have similar temperature requirements per generation (Stacey & Fellowes, 2002). One possible explanation for the displacement of *T. major* during the season is that susceptible *T. major* were killed by spinosad (Tracer) treatments, leaving pesticide-resistant populations of *F. occidentalis* to increase. The increased proportion of *F. occidentalis* associated with increasing number of spinosad (Tracer) treatments observed in this study is supporting evidence for this (section 3.3.2). Spinosad-resistant *F. occidentalis* populations are widespread throughout the world (Sparks *et al.*, 2012) and are known in the UK (Colin Cater, Landseer, pers. comm, 2011). An alternative explanation is that *F. occidentalis* outcompetes *T. major*, as found in Florida, where it has a faster reproductive rate than the native *F. bispinosa* in dense interspecific populations (Northfield *et al.*, 2011). Further studies are required to determine which of these applies.

Low temperatures limited *F. occidentalis* population growth in semi-protected strawberry at the start and end of the season. No population growth was observed before mean temperatures were consistently above 15°C (from mid-May) and thrips numbers declined again at the end of the season (September-October) once mean temperatures dipped below 15°C (Figures 3.1 to 3.3), which is consistent with published data (Gaum *et al.*, 1994). Whilst there was no population growth without sufficient temperature, other factors were important in limiting the speed of growth, the peak thrips numbers and the timing of outbreaks (see below).

The start of population increase in individual crops could not be predicted by temperature alone, as the first larvae were observed on different dates in different crops, between 12 April and 31 May (Figures 3.1 to 3.3). The first occurrence of larvae reflected the timing of first-flowering in second-year crops and the later arrival of thrips into first-year crops. Whilst flowering in strawberry is temperature related (Sønsteby & Heide, 2007), it also reflects cultural practices, such as planting and covering date, plant vigour and whether the grower de-blossoms. The timing of the first larvae in second-year crops was consistent with adults entering flowers as soon as they opened and starting to lay eggs

immediately, as larvae were found 2-3 weeks after the first adults and the development times for eggs is about 10 days at 16°C and a projected 13 days at 11.5°C on strawberry (Nondillo *et al.*, 2008).

Limited data in crops with early-season flowering showed a drop in adult numbers (per flower and per plant) after about four weeks, suggesting that the first generation of adults had died out before the second generation of adults started to emerge (e.g. Figure 3.3. C). In later flowering crops (e.g. from mid-May), there was no clear gap between the first and second generation of adults (e.g. Figure 3.2. C). This could be because of the shorter generation time at warmer temperatures, or because adults are moving in from weeds or other crops. Survival times vary with host plant and temperature and there is no directly comparable published data on strawberry at early-season UK temperatures (11°C to 15°C). Adult females lived about 46 days at 15°C on chrysanthemum (Robb, 1989) and for 21 days at 25°C on strawberry (Nondillo *et al.*, 2009). Further information on the field longevity of *F. occidentalis* on strawberry at different temperatures would improve the prediction of population development in the field.

The rapid increase in thrips numbers observed in July, in most second-year crops (Table 3.3) corresponded with predicted peak emergence of the third generation of adults (using published data from McDonald *et al.* (1998)), by which time there were overlapping generations, average temperatures of about 18°C and optimum daytime temperatures (for development and flight) of 25-30°C. Theoretically, if thrips population development depended upon temperature and rainfall (which can largely be discounted under the polytunnels) alone, adult thrips numbers should have continued to rise from mid-July to the end of the season as there were overlapping generations, more adults emerging daily and because adults emerging in mid-July might survive to near the end of the season at the temperatures recorded. However, the thrips numbers fluctuated around a plateau in all the fields (although at slightly different densities) during July and August that did not relate solely to pesticide treatments (Figures 3.1 to 3.3). As temperatures were ideal for most of the season, thrips populations must have been limited by density-dependent factors during July and August, such as competition for resources (flower availability), reduced oviposition (O'Leary, 2005) and predation (Rahman *et al.*, 2011a).

It was estimated that five generations of *F. occidentalis* could complete their development during the growing season in semi-protected strawberry in the West Midlands

(Table 3.3). A possible sixth generation could be completed after cropping or in warmer areas of the UK. The predicted number of generations was the same, whether using a UK data set (McDonald *et al.*, 1998), or mean values from several data sets (Table 3.3), as fewer day-degrees were required when there was a higher development threshold (see Trudgill *et al.*, 2005). Published development thresholds could not be confirmed, as mean temperatures were mostly below published thresholds before the polythene cladding was in place, but exceeded published thresholds as soon as the cladding was up. As thrips populations increased in different crops at different times (relating to age of crop and flowering time rather than temperature alone), the use of percentage of flowers infested with thrips may be a better predictor of increased thrips density per flower and fruit damage than temperature (Table 3.3), as it would reflect populations specific to each field, but this was not tested. Prediction of thrips damage is discussed further in Chapter 4.

Thrips were caught on traps throughout the cropping period as daytime temperatures exceeded 15°C on most days (Figures 3.1, 3.2, 3.3). This is in line with published information on *F. occidentalis* flight, which showed no take-off at 15°C and increasing flight activity between 20°C-30°C in a UK population (O'Leary, 2005). Few thrips were caught between December and late February (Table 3.5), which could reflect a lower population as well as the low mean (2.5 to 5.5 °C) and maximum (11 to 12.5°C) temperatures during these months (Table 3.5). Correlation between thrips density on plants and trap catch was significant, but variable, as trap catch reflects daily flight pattern, which varies with temperature, wind speed, population density and food availability (Teulon *et al.*, 1999; Pearsall, 2002; O'Leary, 2005; Liang *et al.*, 2010). The comparative use of flower counts or trap catch for assessing thrips populations and predicting fruit damage, and the efficiency of trapping through the season are discussed in Chapters 4 and 5 respectively.

Strawberry flower density varies between cultivars, age of crop and cultural methods and could affect thrips population development. In this study, the highest thrips populations were found in the crops that supported the greatest number of flowers per plant during flower flushes, but there was insufficient replication between crops of the same cultivar to draw conclusions from this. If the number of flowers in a flower flush affects thrips population increase, it could be an important factor that is missing from prediction models (e.g. (Wang & Shipp, 2001)). The number of flowers in a crop is a limiting factor in thrips population growth because egg-laying per female decreases with increasing

numbers of thrips per flower, possibly as a result of interference between individuals within flowers (Kirk, 1994; O'Leary, 2005). The size of flower is also likely to affect the number of thrips within. In this study, the highest count in an individual flower was 82 adult *F. occidentalis* (22 August, 2012, alcohol sample, field 7), although this was an exception, with average numbers peaking at between 4 and 20 adult thrips per flower in different fields. Larger complicated flowers, such as roses, may be able to support more thrips without interference. Andrewartha & Birch (1954) found as many as 400 *T. imaginis* in a rose flower in Australia.

The establishment of the predators *Neoseiulus* spp. in the two crops monitored in 2011 reflected the releases of *N. cucumeris* made by the growers and lower thrips numbers were observed in the crop with more predators. Whilst this is no proof of pest control effect, as there was no replication, it does support published information on strawberry, showing that *N. cucumeris* reduces thrips numbers (Rahman *et al.*, 2012). Very low establishment of *Orius* spp. was observed, which reflected the low temperatures at the time of the release (<15°C), as establishment is poor below 15°C (Cocuzza *et al.*, 1997). One release was made when temperatures were suitable, but it was too late in the season for the predator to exert control. The effect of *N. cucumeris* on fruit damage is tested in Chapter 4.

A wide range of *F. occidentalis* weed hosts were identified from UK strawberry fields (Table 3.4), as found in other crops and in other countries (Chamberlin *et al.*, 1992; Chellemi *et al.*, 1994; Kahn *et al.*, 2005; Katayama, 2006; Jenser, 2008). Weeds were abundant in some crops and as *F. occidentalis* is polyphagous, the total number of weeds could be as important as the prevalence of the most attractive species (Figure 3.5). Three common species that were widespread and flower throughout the year (*S. media*, *S. vulgaris* and *T. officinale*) supported overwintering populations of *F. occidentalis* (Table 3.4), so contributing to the carry-over of thrips from first to second-year crops and were a source of infestation in first-year crops. Further studies are required to quantify the impact of weed hosts on thrips abundance in strawberry, but whether they increase local thrips abundance, cause an invasion of thrips onto crops when controlled (Allsopp, 2010), or support predator populations (Frescata & Mexia, 1996; Honek *et al.*, 2005), they need careful management.

Overwintering of adult female *F. occidentalis* was confirmed in strawberry and weed flowers in the Midlands, UK (Table 3.4), which contrasts with controlled studies, where *F.*

occidentalis failed to overwinter in the same region (McDonald *et al.*, 1997b). As air temperatures were broadly similar between the two studies, it is possible that the ‘wild’ populations are able to survive by moving into protected micro-climates, such as inside weed flowers or under the plastic mulch used to cover strawberry beds, which can increase soil temperatures by several degrees (Diaz-Perez *et al.*, 2007). The use of polytunnels (e.g. from April to October) could make the difference as they effectively shorten the winter, and some adults in the study by McDonald *et al.* (1997b) nearly made it through the winter, the longest-lived surviving for nearly 90 days. Males apparently died out over winter and they are known to be shorter-lived than females in cold conditions (Tsumuki *et al.*, 2007). As temperatures are predicted to increase by between 1 to 3.5°C by 2100 (Cannon, 2004), it is likely that the distribution of *F. occidentalis* will expand further northwards as a result (Krumov & Karadjova, 2012). To date, there is conflicting evidence of local adaption to cooler climates, and a UK study found no difference in temperature requirements between *F. occidentalis* collected from the field near northern and southern research stations (Stacey & Fellowes, 2002). However, the thrips collected in their study may have originated from related glasshouse cultures, so it would be interesting to compare the development thresholds of thrips collected from strawberry fields in the spring, from Kent, Staffordshire and Angus (i.e south, middle and north range of latitudes).

The overwintering of thrips could be a critical factor in the management of thrips, as the lower numbers of *F. occidentalis* in first-year crops are usually well controlled by the predator *N. cucumeris* (e.g. Figure 3.7 A). Growers could reduce thrips risk on their farms by growing only one-year crops, using good weed control or by identifying treatments that effectively reduce the overwintering thrips population.

The widespread distribution and abundance of *F. occidentalis* in second-year crops, compared to first-year crops, at first flowering in April is supporting evidence that the thrips have over-wintered in the crops. In some second-year crops there were sufficient thrips in the first generation to cause crop damage very early in the season (e.g. >4 thrips per flower, Figure 3.3. A). Maximum temperatures only exceeded 20°C (when flight becomes more frequent) occasionally in April, so the thrips are unlikely to have flown into these crops from outside. As no thrips were found in 100 flowers in three first-year crops sampled at first flowering in April and about 74% of the adult thrips population are found in flowers (see Chapter 4), it suggests that the thrips populations in second-year crops develop mostly from resident thrips. When the thrips arrived in first-year crops, they were

widespread throughout the crop (although a slight bias towards the edges for the first month) and could have come from several sources: Thrips can be brought in on incoming plants (Kirk & Terry, 2003), could enter the crop from weeds (Table 3.3), or on farm equipment (picking trays, boxes, tractors) and staff, who move daily from field to field, or can fly in from nearby fields once temperatures are suitable. The frequent movement of thrips between strawberry flowers (Table 3.5) helps to explain how the thrips spread through strawberry crops and why they are usually found throughout affected crops. Even if the thrips enter from a weedy field margin and spread slowly at the 0.3 m per day observed in glasshouse crops (Rhainds & Shipp, 2004), they would spread through most strawberry fields by the end of the season. Spread through open-sided polytunnels is likely to be accelerated by wind currents. It would be useful to measure thrips entrance and departure rates from flowers at different temperatures and different thrips densities to understand how thrips density affects movement and therefore spread through the crop (Crespi & Taylor, 1990). Further data are required to determine the amount of movement between fields.

Within fields, the higher thrips numbers in the top to middle of sloping fields reflected the higher mean and daytime temperatures in those areas (Figures 3.8, 3.9, 3.10), which would result in an increased reproductive rate (Robb, 1989). In addition, when the sun comes out, warm air currents move up the tunnels possibly carrying thrips with them. Individual fields may have other influences and specific hot-spots (e.g. adjacent crops, prevailing wind, weed control, spraying etc.), but tunnel temperature has a significant effect on thrips numbers and this information can be used by growers to predict areas within crops that are most at risk from thrips damage.

This study has identified a number of factors that could be manipulated by growers to improve control. *Frankliniella occidentalis* was confirmed as the dominant thrips species in the semi-protected strawberry crops sampled and the thrips overwintered on strawberry and weeds within the fields. As adult females were found in senescent/dead strawberry flowers and weed flowers throughout the winter, one control option might be to remove these at the end of cropping. Other treatments could be identified that target the overwintering thrips population. As the carry-over of thrips to second-year crops was identified as a risk factor, growers could consider one-year crops in fields with high thrips populations in order to reduce thrips populations. Temperature gradients within polytunnels explained some local variations in thrips abundance, and this information

could be used by growers to target areas most at risk from thrips damage, for sampling and control. Factors affecting the timing and abundance of thrips populations were identified, but controlled studies are required to test how these factors can be manipulated within integrated pest management programmes.

Table 3.1. Lower development thresholds reported in the literature (in °C) and the total thermal requirement (in day-degrees) above the development threshold required to complete a generation (egg to adult) of *F. occidentalis*.

| Reference | Lower development threshold (°C) | Day-degree requirement (°C) | Host plant |
|--|--|--------------------------------|---------------|
| Bryan & Smith (1956) | 9.4 | 238.1 | Radish |
| Lublinkhof & Foster (1977) | 5.3 | 303.0 | Green beans |
| Sites & Chambers ¹ (1990) | 6.7 | 114.0 | Alfalfa |
| Robb & Parrella (1991) | 11.8 | 169.5 | Chrysanthemum |
| Lowry <i>et al.</i> (1995) | 6.5 | 253.9 | Peanut |
| Gaum <i>et al.</i> (1994) | 9.4 | 249.8 | Cucumber |
| Katayama (1997) | 9.5 | 194.0 | Chrysanthemum |
| Jarošík <i>et al.</i> (1997) | 10.7 | 231.1 | Cucumber |
| McDonald <i>et al.</i> ² (1998) | 7.9 | 268.0 | Chrysanthemum |
| Stacey & Fellowes ² (2002) | 6.7 | 233.4 | Green beans |
| Gitonga <i>et al.</i> (2002) | 9.0 | 256.8 | Green beans |
| Nondillo <i>et al.</i> ³ (2008) | 9.9 | 211.9 | Strawberry |
| Zhang <i>et al.</i> (2012) | 7.8 | 208.0 | Green beans |
| Mean ± SEM | 8.5 ± 0.5 | 225.5 ± 13.3 | |

¹ USA population, wild-collected in spring from alfalfa

² UK populations

³ Brazilian population, from strawberry

Table 3.2. Thrips control treatments applied to semi-protected strawberry crops monitored for seasonal thripid abundance during 2011 and 2012. Field numbers refer to Table 2.1.

| Week no. | Thrips control treatments | | | |
|----------|--|---|--|---|
| | Field 2, 2011 cv. Camarillo | Field 3, 2011 cv. Camarillo | Field 5, 2012 cv. Finesse | Field 7, 2012 cv. Finesse |
| 13 | | <i>S. scimitus</i> 250 per m ² | | <i>N. cucumeris</i> bags 1 per 2 m of bed |
| 18 | <i>N. cucumeris</i> 50 per m ² | <i>N. cucumeris</i> bags 1 per 2 m of bed | | <i>N. cucumeris</i> 250 per m ² , fortnightly |
| 19 | Spinosad (Tracer) | | | |
| 20 | <i>N. cucumeris</i> 50 per m ² | Heat treatment | | |
| 23 | | <i>O. laevigatus</i> 0.5 per m ² | <i>N. cucumeris</i> 250 per m ² fortnightly | Spinosad (Tracer) |
| 26 | | Heat treatment | Abamectin (Acaramite) | <i>N. cucumeris</i> 500 per m ² weekly |
| 27 | | <i>N. cucumeris</i> 160 per m ² | | Spinosad (Tracer) |
| 29 | Spinosad (Tracer) | Maltodextrin (Eradicoat) <i>N. cucumeris</i> 400 per m ² | | |
| 30 | | Spinosad (Tracer) | | <i>B. bassiana</i> (Naturalis) |
| 31 | Spinosad (Tracer) | <i>O. laevigatus</i> (2.4 per m ²) <i>N. cucumeris</i> (300 per m ²) | | |
| 35 | | <i>N. cucumeris</i> (200 per m ²) | | |
| 36 | Spinosad (Tracer) | Spinosad (Tracer) | Spinosad (Tracer) Abamectin (Acaramite) <i>B. bassiana</i> (Naturalis) | Spinosad (Tracer) |

Table 3.3. The number of day-degrees above development thresholds of 7.9°C (McDonald *et al.*, 1998) or 8.5°C (mean from Table 3.1) and the estimated maximum number of generations that could complete development up to the end of cropping in 2011 and 2012, based on temperature records taken from within the canopy of semi-protected strawberry crops in the West Midlands, UK; the timing of maximum increase in thrips adult numbers (mean numbers per plant/mean numbers per plant in the previous week); the timing of 100% flower occupation by thrips. Field numbers refer to Table 2.1.

| | Field 2, 2011 | Field 3, 2011 | Field 5, 2012 ^a | Field 7, 2012 ^a |
|---------------------------------------|------------------------------|---------------|----------------------------|----------------------------|
| McDonald data ^b | | | | |
| Day degrees | 1368 | 1329 | 1423 | 1423 |
| Generations | 5.1 | 5.0 | 5.3 | 5.3 |
| Mean data from Table 3.1 ^c | | | | |
| Day degrees | 1180 | 1159 | 1214 | 1214 |
| Generations | 5.5 | 5.4 | 5.6 | 5.6 |
| Timing of maximum increase | 19 July | 26 July | 29 May | 9 July |
| Date of 100% flower occupancy | 2 August (80% on 19 July) | 26 July | 29 May | N/A ^d |

^aNearby fields on the same farm used the same temperature data.

^b268 degree-days above a threshold of 7.9°C

^c226 degree-days above a threshold of 8.5°C

^dThis first-year crop did not reach 100% thrips occupancy of flowers

Table 3.4. Weed host-plants that were identified from a semi-protected strawberry field (field 3, Table 2.1) on 28 October 2011.

Key: * = Weed species that flower throughout the year and were present in every tunnel in all the fields in Table 2.1; a = less than five adult *F. occidentalis* per sample; A = five or more adult *F. occidentalis* per sample; l = less than five thripid larvae per sample; L = five or more thripid larvae per sample; - = none found.

| Weed host family and species | Main flowering period (months) | Presence of adult <i>F. occidentalis</i> | Presence of thripid larvae |
|--|--------------------------------|--|----------------------------|
| Apiaceae | | | |
| <i>Heracleum sphondylium</i> | 6-9 | a | l |
| Asteraceae | | | |
| <i>Matricaria discoidea</i> | 6-7 | a | L |
| * <i>Senecio vulgaris</i> | 1-12 | a | L |
| <i>Sonchus asper</i> | 6-8 | a | l |
| * <i>Taraxacum officinale</i> | 1-12 | A | L |
| <i>Tripleurospermum inodorum</i> | 7-9 | A | L |
| Brassicaceae | | | |
| <i>Brassica rapa</i> Ssp <i>oleifera</i> | 5-8 | - | - |
| <i>Capsella bursa-pastoris</i> | 6-9 | - | - |
| <i>Sisymbrium officinale</i> | 6-7 | a | - |
| Caryophyllaceae | | | |
| <i>Cerastium glomeratum</i> | 4-9 | a | l |
| * <i>Stellaria media</i> | 1-12 | a | l |
| Fabaceae | | | |
| <i>Trifolium repens</i> | 6-9 | - | - |
| Poaceae | | | |
| <i>Poa annua</i> | 1-12 | a | - |
| Rubiaceae | | | |
| <i>Galium aparine</i> | 6-8 | a | l |
| Solanaceae | | | |
| <i>Solanum nigrum</i> | 7-9 | A | l |
| Urticaceae | | | |
| <i>Urtica dioica</i> | 6-8 | a | - |
| Verbenaceae | | | |
| <i>Verbena officinalis</i> | 7-9 | a | l |
| Veronicaceae | | | |
| <i>Veronica persica</i> | 1-12 | a | l |

Table 3.5. Overwintering of *F. occidentalis* in UK strawberry.

The presence or absence of adult *F. occidentalis* and thripid larvae on overwintered strawberry plants and selected weed species flowers, the mean trap catch \pm SEM with or without pheromone on blue sticky traps, and mean, maximum and minimum overwinter temperatures in semi-protected strawberry crops in the W. Midlands, UK.

Key: A = adult *F. occidentalis* (males and females), F = female *F. occidentalis*, L = thripid larvae.

| <i>Total number of F. occidentalis adults and thripid larvae found on flower samples^a</i> | | | | | |
|--|--------------------------|--------------------------|--------------------------|--------------------------|----------------------------|
| Date | 29 Nov 2011 | 13 Dec 2011 | 25 Jan 2012 | 22 Feb 2012 | 22 March 2012 |
| Strawberry | 5 A, 6 L* | 2 A, 4 L* | 1 F, 0 L | 0 | 2 F, 0 L |
| Groundsel | 2 A, 11 L* | 1 F, 2 L* | 1 F, 0 L* | 0* | 1 F, 0 L* |
| Dandelion | 13 A, 2 L* | 4 A, 2 L* | 1 F, 0 L* | 1 F, 0 L* | 0* |
| Chickweed | 1 F, 2 L* | 2 A, 0 L* | 0 ^b | 0 ^b | 3 F, 0 L* |
| <i>Mean trap catch (n = 6 traps)</i> | | | | | |
| Date | 29 Oct to 29 Nov 2011 | 29 Nov to 13 Dec 2011 | 13 Dec to 25 Jan 2012 | 25 Jan to 22 Feb 2012 | 22 Feb to 22 March 2012 |
| Blue sticky | 1.4 A \pm 0.5 | 0.2 A \pm 0.2 | 0 | 0 | 5.8 F \pm 1.5 |
| Blue sticky pheromone | 5.0 A \pm 2.5 | 0.5 A \pm 0.3 | 0.2 F \pm 0.2 | 0.2 F \pm 0.2 | 14.2 F \pm 7.3 |
| <i>Temperature (°C)</i> | | | | | |
| Maximum | 15.5 | 12.5 | 11 | 12.5 | 25.5 |
| Mean | 9.3 | 5.4 | 5.5 | 2.5 | 8.6 |
| Minimum | 4 | 1 | -4.5 | -6 | -1.5 |

^a n = 15 strawberry, 9 dandelion, 30 groundsel or 30 chickweed flowers. Where open flowers were not available, senescent or dead flowers were collected.

*Open flowers were available

^b Chickweed leaves had silver feeding marks and faecal deposits typical of *F. occidentalis*.

Table 3.6. Number of adult thrips per flower in ten marked flowers, sampled hourly from 10.00 h to 13.00 h. The data shows that the number of adult thrips within individual flowers differed from the previous count in 24 out of 30 occasions, as indicated by (+, -) for an increase or decrease and (=) where no change was observed, showing frequent movement between flowers. Individual flowers differed in the number of thrips that they supported ($F_{(9, 30)} = 11.6$, $P < 0.001$) but the number of thrips per flower was not significantly different between sample times ($F_{(3, 36)} = 0.7$, $P = 0.6$). The table shows untransformed means whereas the statistical analysis used log transformed data.

| Flower number | Adult thrips per flower at different sampling times | | | | Mean \pm SEM per flower |
|----------------|---|--------------------|--------------------|--------------------|---------------------------|
| | 10.00 am 17.6°C | 11.00 am 21.4°C | 12.00 pm 19.7°C | 13.00 pm 24.6°C | |
| 1 | 6 | 4 (-) | 2 (-) | 5 (+) | 4.5 \pm 0.9 |
| 2 | 1 | 1 (=) | 0 (-) | 1 (+) | 0.8 \pm 0.3 |
| 3 | 1 | 0 (-) | 1(+) | 0 (-) | 0.5 \pm 0.3 |
| 4 | 0 | 1 (+) | 1 (=) | 0 (-) | 0.5 \pm 0.3 |
| 5 | 4 | 1 (-) | 1 (=) | 2 (+) | 2.0 \pm 0.7 |
| 6 | 2 | 1 (-) | 2 (+) | 2 (=) | 1.8 \pm 0.3 |
| 7 | 6 | 6 (=) | 4 (-) | 6 (+) | 5.5 \pm 0.5 |
| 8 | 4 | 3 (-) | 2 (-) | 3 (+) | 3.0 \pm 0.4 |
| 9 | 5 | 4 (-) | 3 (-) | 4 (+) | 4.0 \pm 0.4 |
| 10 | 3 | 1 (-) | 1 (=) | 2 (+) | 1.8 \pm 0.5 |
| Mean \pm SEM | 3.2 \pm 0.7 | 2.2 \pm 0.6 | 1.7 \pm 0.4 | 2.5 \pm 0.6 | 2.4 \pm 0.3 |

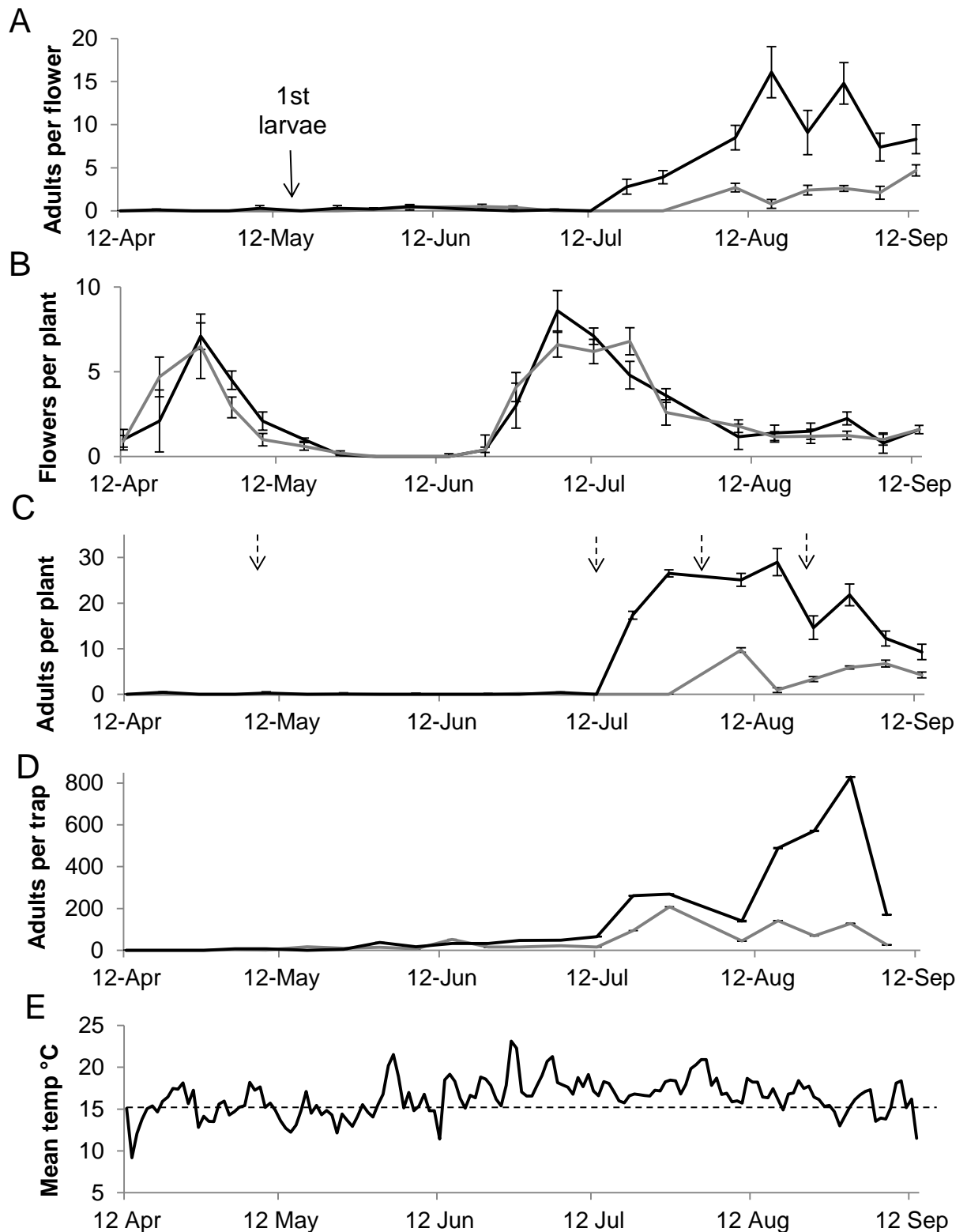


Figure 3.1. Seasonal changes in (A) adult thrips per flower, (B) flowers per plant, (C) adult thrips per plant (thrips per flower \times flowers per plant), (D) adult thrips per blue sticky pheromone trap and (E) mean temperature ($^{\circ}\text{C}$) in two plots in a second-year semi-protected strawberry crop (cv. Camarillo) (field 2, Table 2.1) in 2011. \downarrow = spinosad applied.

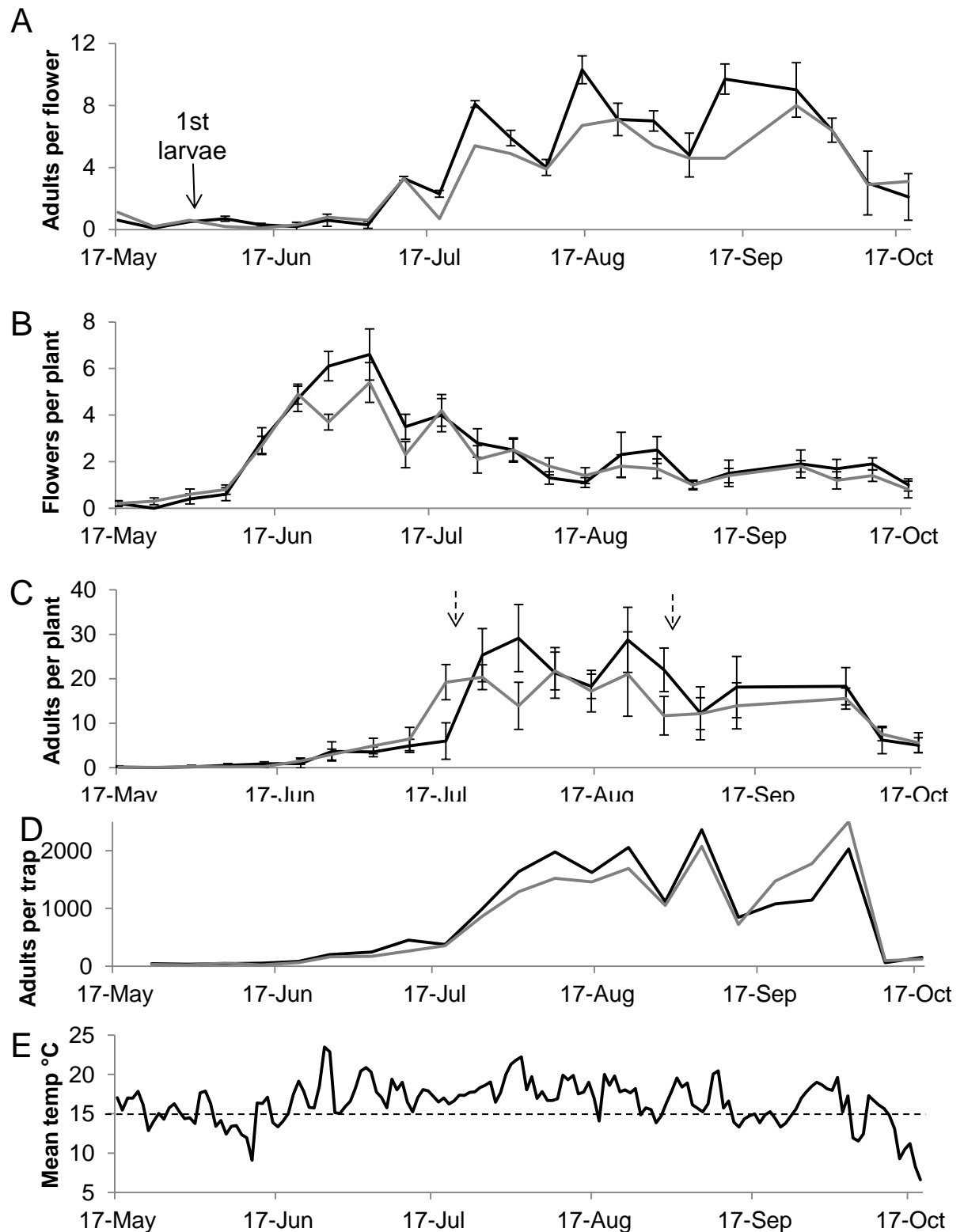


Figure 3.2. Seasonal changes in (A) adult thrips per flower, (B) flowers per plant, (C) adult thrips per plant (thrips per flower \times flowers per plant), (D) adult thrips per blue sticky pheromone trap and (E) mean temperature ($^{\circ}\text{C}$) in two plots in a second-year semi-protected strawberry crop (cv. Camarillo) (field 3, Table 2.1) in 2011. \downarrow = spinosad applied.

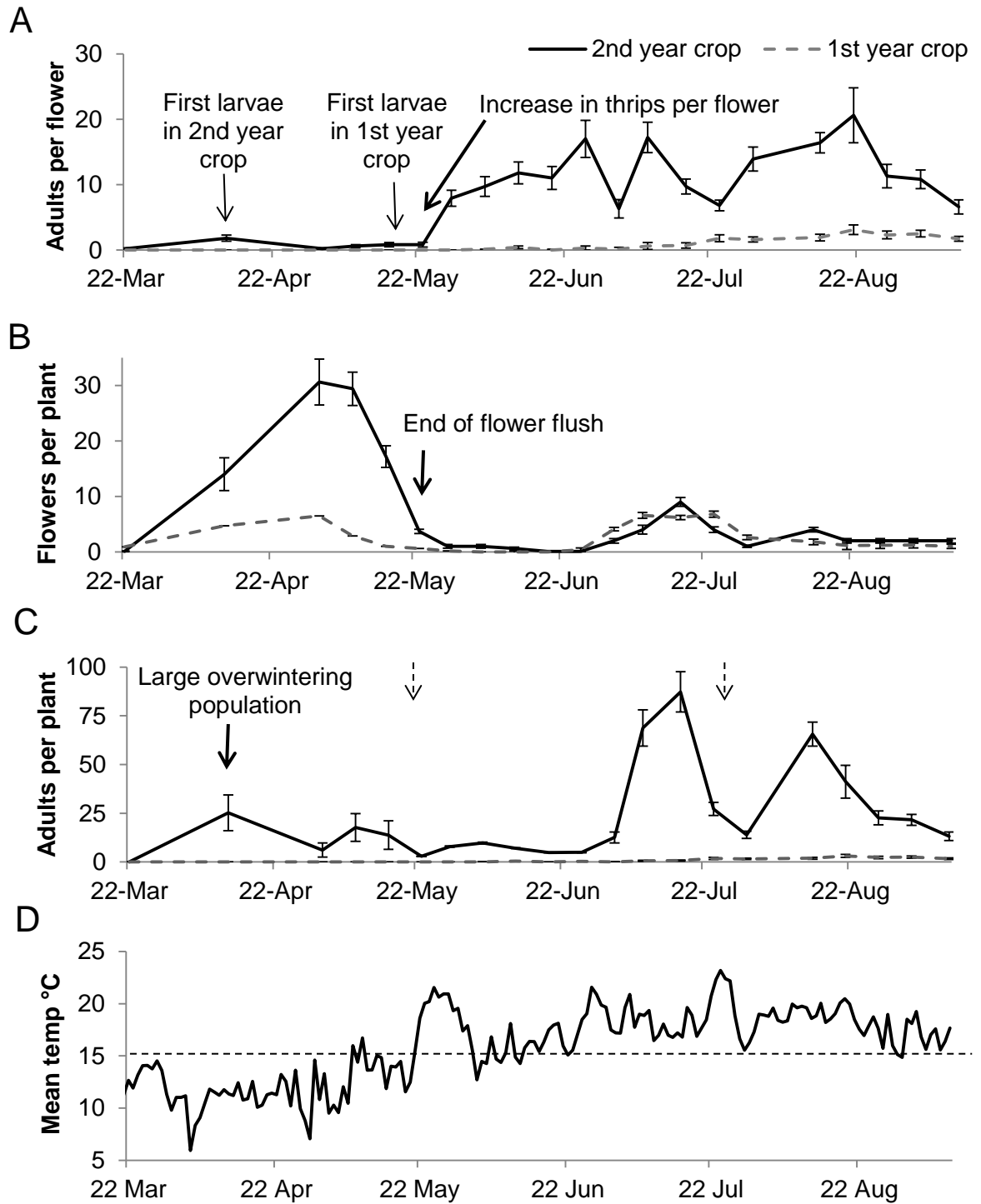


Figure 3.3. Seasonal changes in (A) adult thrips per flower, (B) flowers per plant, (C) adult thrips per plant (thrips per flower \times flowers per plant), and (D) mean temperature ($^{\circ}\text{C}$) in two plots in first-year (cv. Finesse, field 5, Table 2.1) and second-year (cv. Finesse, field 7, Table 2.1), semi-protected strawberry crops in 2012. ∇ = spinosad applied.

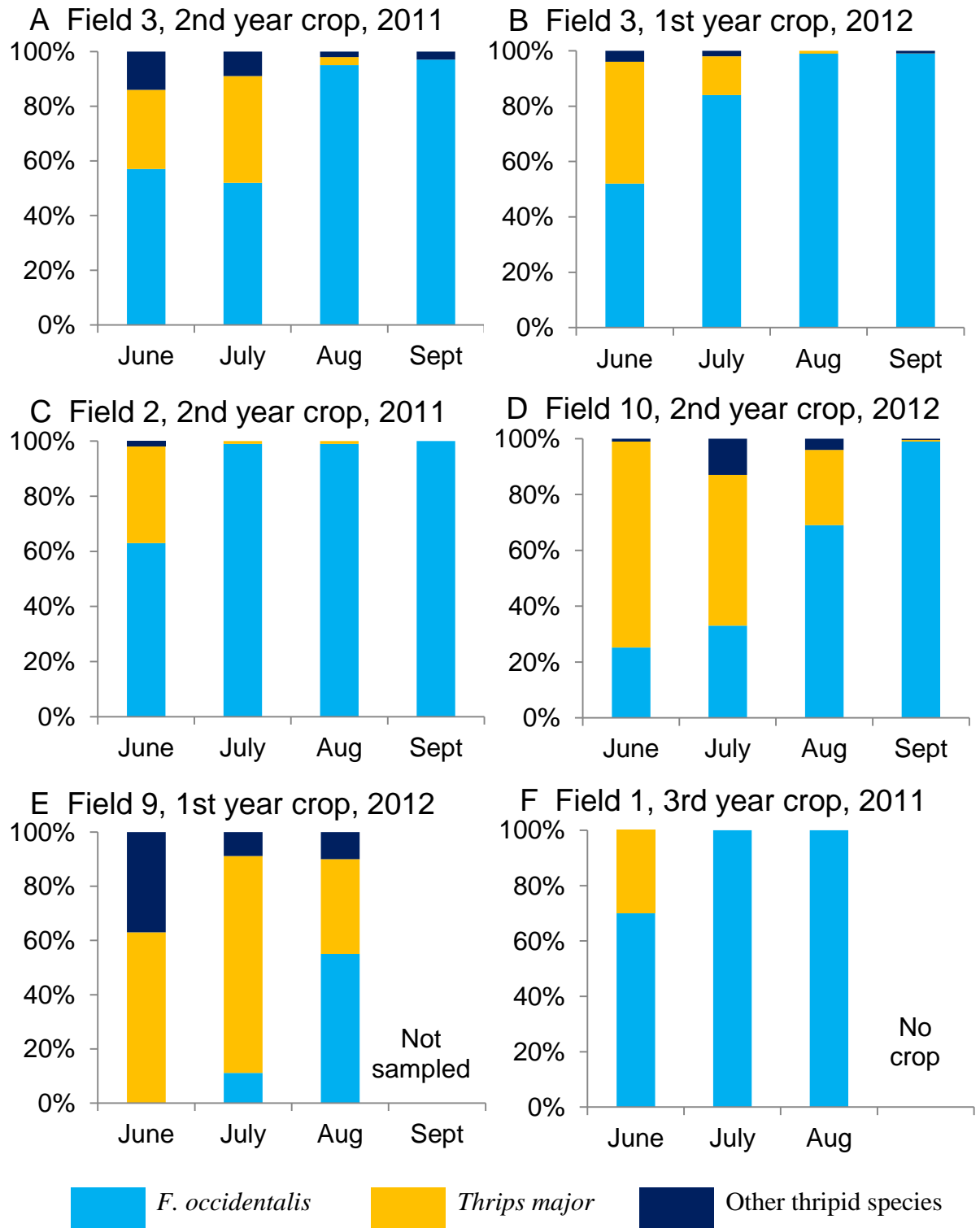


Figure 3.4. Thrips species composition through the season in semi-protected strawberry fields in the West Midlands, UK, 2011 and 2012. The percentage of adult thrips in strawberry flowers that were *Frankliniella occidentalis*, *Thrips major* or other species is shown ($n > 100$ for all samples).

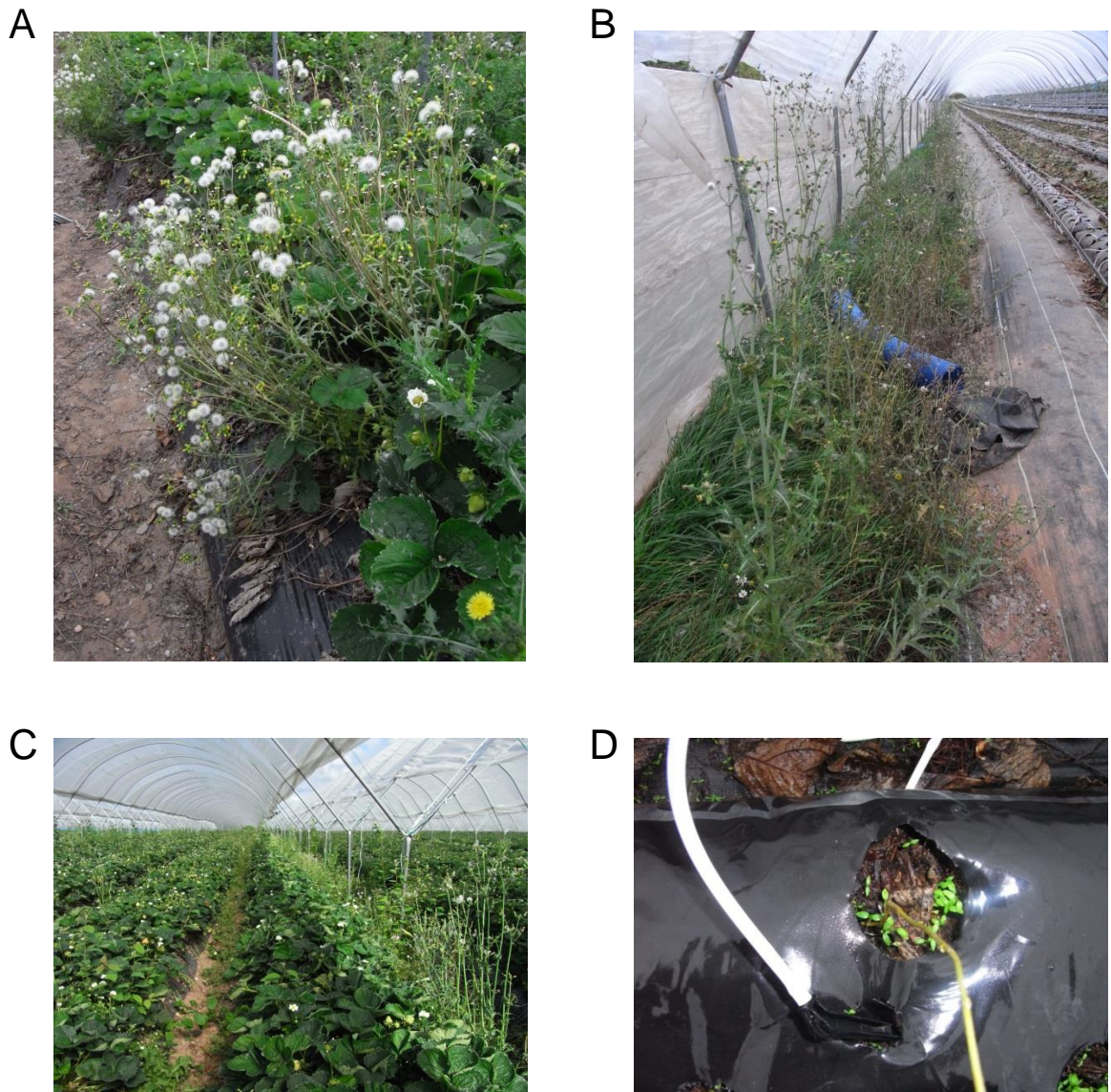


Figure 3.5. Common *F. occidentalis* weed hosts in semi-protected strawberry.

(A) *Senecio vulgaris* and *Taraxacum officinale*, (B) a mix of weed species along field margins and, (C) between polytunnel rows, (D) *Stellaria media* seedlings growing in an empty growbag in January.

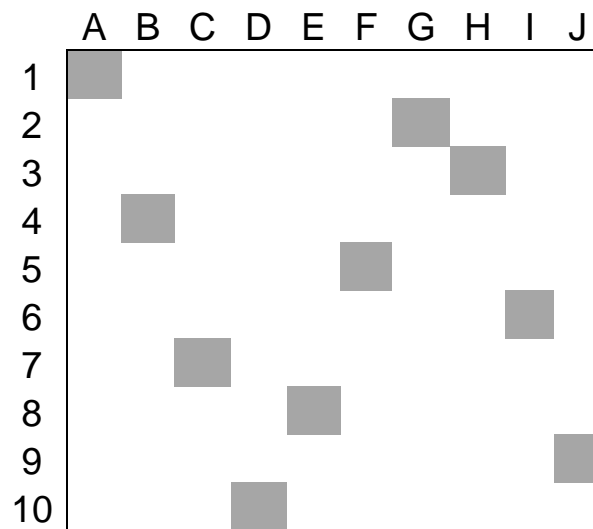


Figure 3.6. Sampling plan adopted for assessing the distribution and abundance of thripids in three first-year and three second-year semi-protected strawberry crops through the season (section 3.2.5). The length (1 to 10) and breadth (A to J) of each crop was divided into ten equal sections and plots distributed as shown in grey. Ten flowers were sampled from each of the marked plots (n= 100 flowers and fruit per crop).

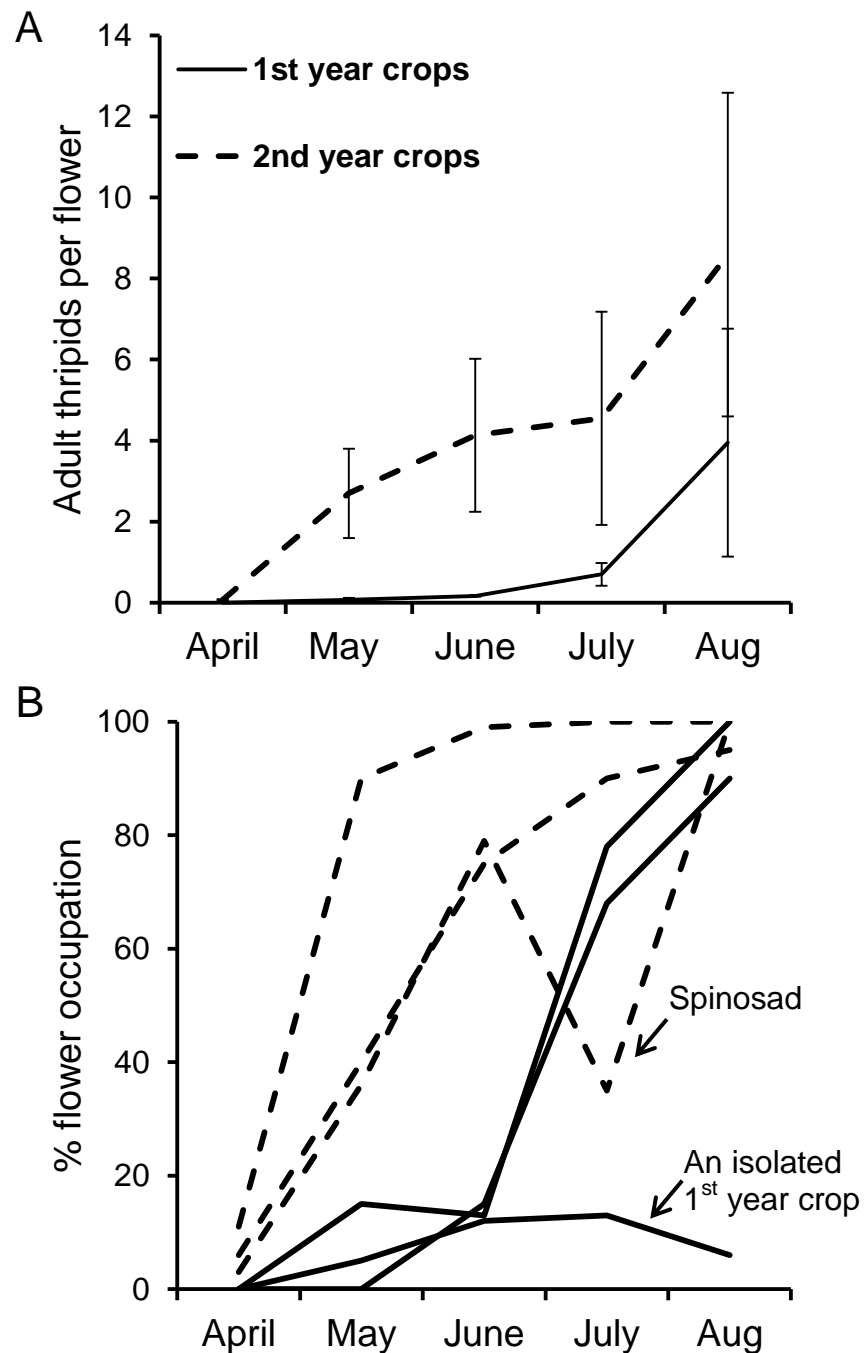


Figure 3.7. (A) Mean adult thrips per flower \pm SEM from samples taken in April, May, June, July and August from first and second-year, semi-protected strawberry crops (cvs. Camarillo and Finesse, $n = 3$ fields of each cultivar). Differences are significant from April to June, but not in July and August (April, $P = 0.04$; May, $P = 0.01$; June, $P = 0.02$; July, $P = 0.18$; Aug, $P = 0.32$). Analysis was on log-transformed data whilst the chart shows untransformed data. (B) The percentage of flowers occupied by thrips in the three first-year and three second-year crops sampled, through the season ($n = 100$ flowers).

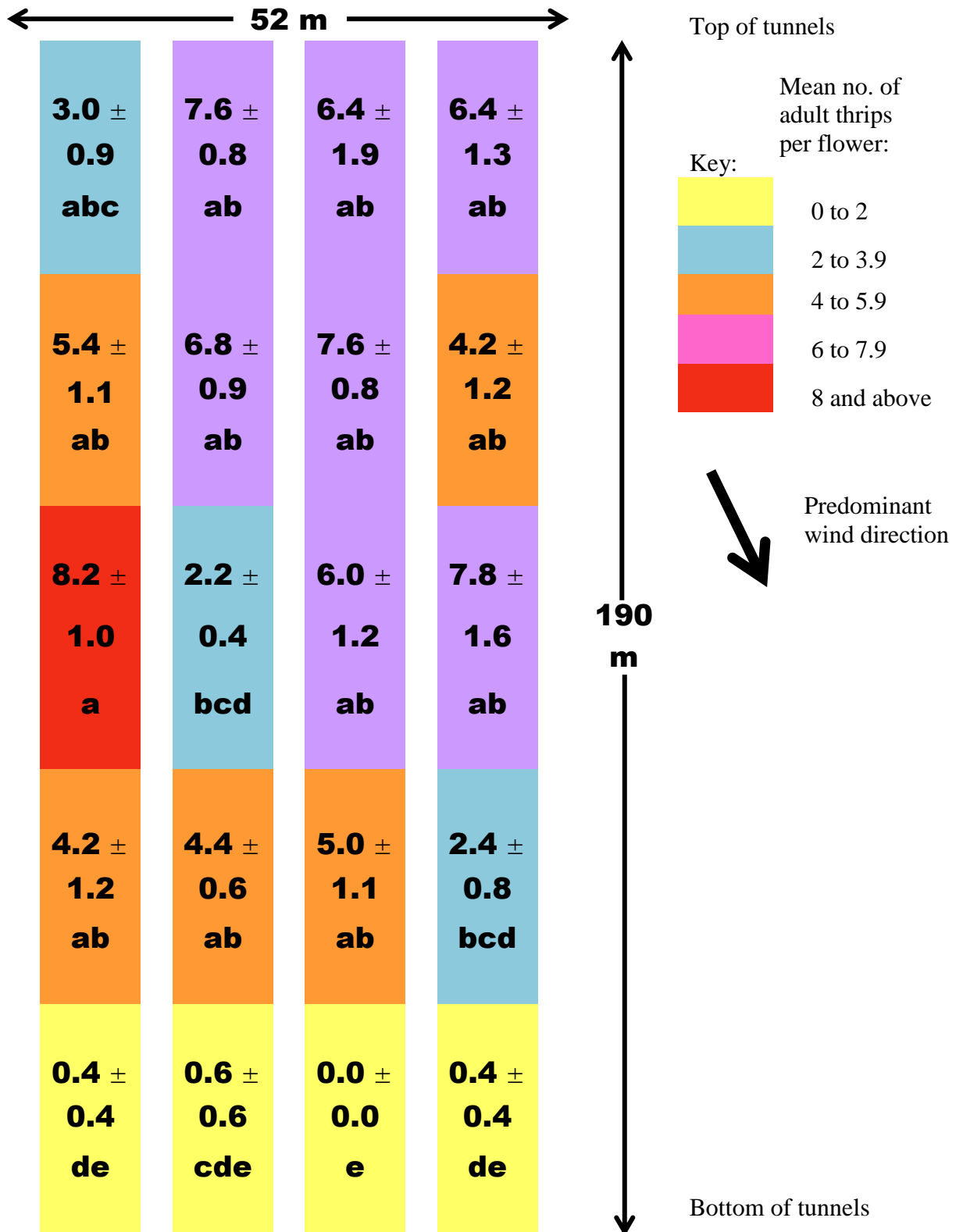


Figure 3.8. The distribution of thrips in a second-year, semi-protected strawberry crop (cv. Finesse) on 6 June 2012 (field 7, Table 2.1), showing the mean number \pm SEM of adult thripids per flower ($F_{(19, 80)} = 12.3$, $P < 0.001$) ($n = 5$ flowers per sample) at different distances down four (out of 8) tunnels. Means with the same letter are not significantly different ($P > 0.05$).

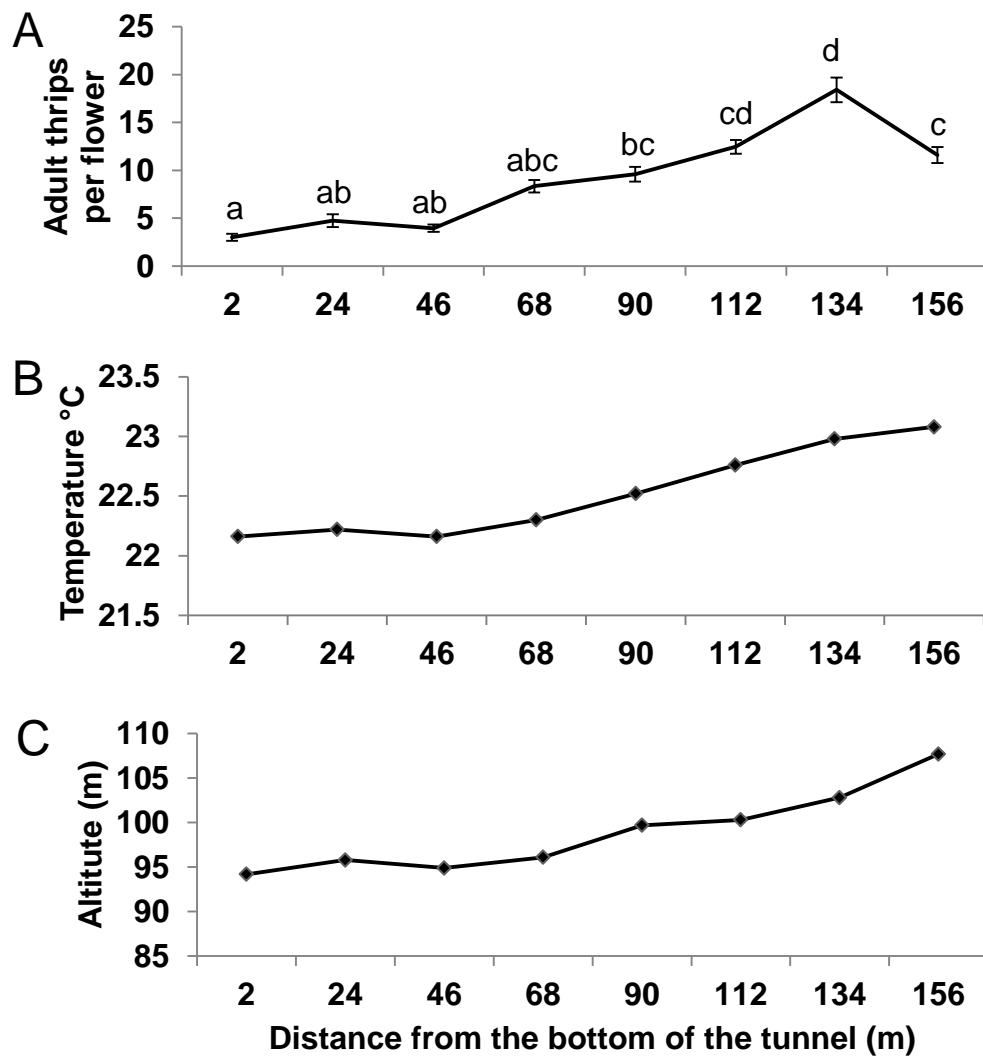


Figure 3.9. The distribution of thrips in a second-year, semi-protected strawberry crop (cv. Camarillo) on 13 September 2012 (field 4, Table 2.1) showing, (A) the mean number \pm SEM of adult thripids per flower ($F_{(7, 80)} = 13.8$, $P < 0.001$) ($n = 5$ flowers per sample, 4 tunnels), (B) Mean temperature ($n = 4$ tunnels) and (C) mean altitude at different distances up the tunnels ($n = 4$ tunnels). Means with the same letter are not significantly different (Tukey's test, $P > 0.05$). Analysis was on log-transformed data whilst the charts show untransformed data.

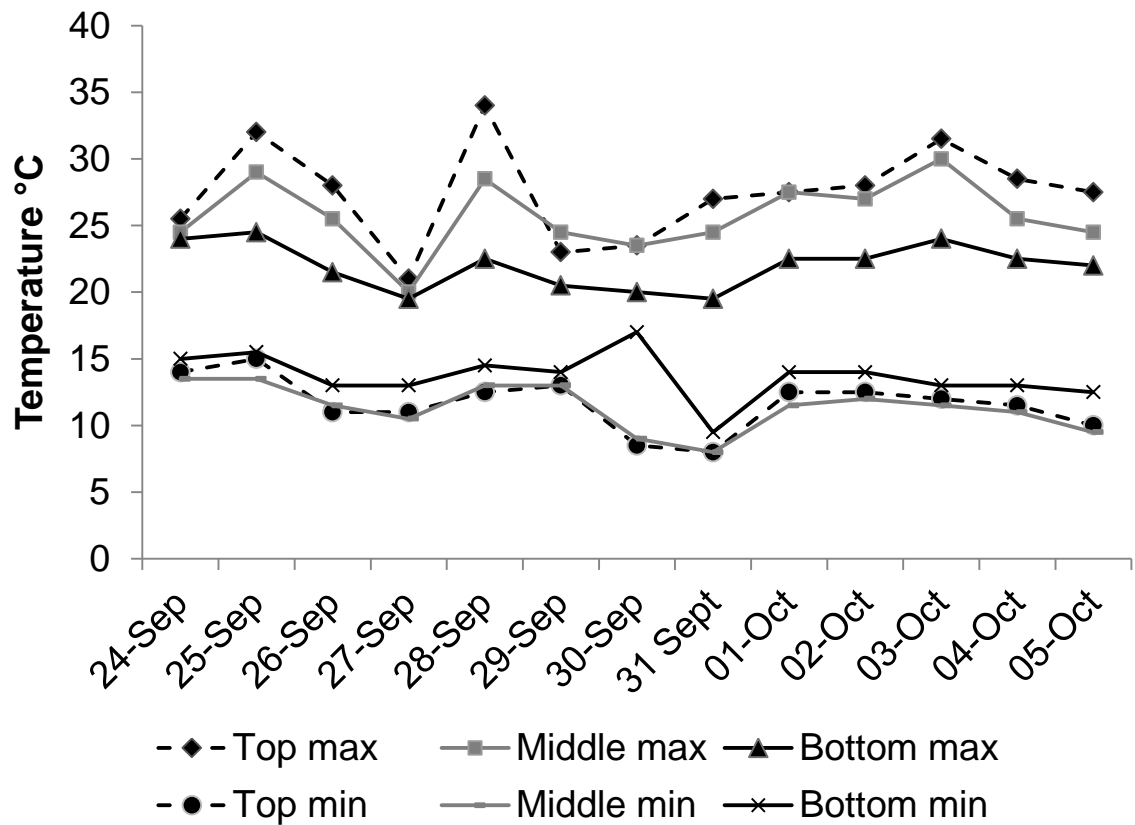


Figure 3.10. The daily maximum and minimum temperatures (°C) at top (107 m altitude), middle (101 m altitude) and bottom (95 m altitude) positions in a semi-protected strawberry tunnel (field 4, Table 2.1) from 24 September to 5 October 2012. Temperatures were recorded using data loggers suspended between the crop canopy at about flower height in white delta traps to shade them from direct sunlight.

Chapter 4

Damage to strawberry fruit

4.1. Introduction

Strawberry is a high-value crop in the UK with a production value of about £222 million in 2012 (Department for Environment, Food and Rural Affairs, 2013). About 4,648 ha were grown in 2012 (Department for Environment, Food and Rural Affairs, 2013), of which about one third were susceptible, everbearer varieties (continuously flowering and fruiting over several months) grown under open-sided polytunnels. In such crops, 10-15% losses due to thrips damage are typical (R. Harnden, pers. comm., 2013) as a result of increasing resistance to chemical insecticides (Sparks *et al.*, 2012). This 10-15% over 1550 ha equates to about £7-11 million loss to the producer due to *F. occidentalis* per annum in UK strawberry.

On strawberry, the most important damage due to *F. occidentalis* is bronzing (russetting) on fruit, which correlates directly with thrips density in flowers (Katayama, 2005; Steiner & Goodwin, 2005a; Coll *et al.*, 2007a). Damage can appear on very young fruit as a result of thrips feeding on the tissue between the seeds (Figure 4.1 A), resulting in a net-like pattern as the fruit matures (Figure 4.1 B) (Steiner & Goodwin, 2005a; Nondillo *et al.*, 2010). When thrips feed on mature fruit they feed in the seed-pits causing bronzing close to the seed (Figures 4.1 C, D). Slight damage to the fruit appears as tracking around the calyx and small patches of bronzing around a few seeds. High numbers of thrips result in punctured, bronzed and small fruit which are dull in appearance, which have a limited shelf life and are unmarketable (Figure 4.1 E, Figure 4.2 B) (Steiner & Goodwin, 2005a). The limited data on the susceptibility of different strawberry stages to bronzing are contradictory (Coll *et al.*, 2007a; Nondillo *et al.*, 2010) and further data are needed to identify the timing of damage so that growers can take timely action to prevent damage. Flower damage can be economically damaging when thrips numbers are high (e.g. >25 thrips per flower) (Steiner & Goodwin, 2005b; Coll *et al.*, 2007a). Symptoms in the flower include necrotic and withered anthers and calyces, flower and fruitlet abortion, reduced

receptacle size, petal browning and premature petal drop (Figure 4.2 A) (González-Zamora & Garcia-Marí, 2003; Coll *et al.*, 2007a; Atakan, 2008; Nondillo *et al.*, 2010). Bronzing symptoms are exacerbated by hot, dry conditions and can be reduced by some cultural methods such as misting, choice of mulch or high drip-irrigation rates (Larson *et al.*, 2004; Steiner & Goodwin, 2006). Some strawberry cultivars are more tolerant than others to thrips damage (Kitamura & Kashio, 2004), or more favourable for *F. occidentalis* population growth (Rahman *et al.*, 2010), so the relationship between thrips density and bronzing damage needs to be quantified under UK conditions on cultivars used by UK growers to develop damage thresholds for the UK.

Several causes of strawberry bronzing are recognised that may be confused with thrips damage, including strawberry tarsonemid mite (*Phytonemus pallidus*), chemical spray burn, sun scorch and hot, dry conditions (Polito *et al.*, 2002). Damage caused by hot, dry weather usually results in a more even distribution of bronzing over the fruit than that caused by thrips and bronzing from spray or sun-scorch is limited to exposed areas of the fruit. Early reports suggested that the feeding on styles and stigmas by thrips could cause malformation of strawberry fruit (“cat-facing”) (Allen & Gaede, 1963; Buxton & Easterbrook, 1988), but some experimental work refutes this (Easterbrook, 2000; Atakan, 2008; Nondillo *et al.*, 2010). Cat facing is a symptom of *Lygus* spp, which occurs at very low pest numbers and may be confused with thrips damage in the field (Easterbrook & Simpson, 2000). Fruit distortion is affected by cultivar and increases with poor pollination and low temperatures (Ariza *et al.*, 2012), indeed, *F. occidentalis* could reduce fruit distortion by contributing to pollination at times of poor fruit set (Kirk, 1988). Another possible benefit of low densities of *F. occidentalis*, not investigated here, is that they predate spider mites, *Tetranychus urticae* (Pickett *et al.*, 1988; Steiner & Goodwin, 2006).

To control pesticide-resistant *F. occidentalis*, growers need to know what density of thrips can be tolerated without economic damage so that unnecessary sprays can be avoided, or when timely actions should be taken to prevent damage. Economic injury levels (EILs) were originally defined as the lowest population density of a pest that will cause economic damage (Stern *et al.*, 1959). Economic damage thresholds (the amount of damage that justifies the cost of artificial control) include calculations for the cost and effectiveness of specific control measures (Pedigo *et al.*, 1986), but these cannot be included when the resistance level of the pest is unknown, as treatment could result in an increase in pest numbers if predator numbers are reduced but not thrips. Action thresholds

(ATs) are the pest density at which control measures should be implemented to prevent it from reaching the EIL. ATs were beyond the scope of this study as they vary according to the treatment being considered, for example, biopesticides typically have a longer lead time than chemical insecticides (as they take longer to work) and the predatory mite *N. cucumeris*, which can survive and reproduce on pollen, should usually be established preventatively before thrips numbers increase. Damage thresholds (DTs) may be defined as the lowest population density that will cause measurable damage. As one *F. occidentalis* can cause measurable (but not economic) damage in strawberry, the use of this measure to time insecticide treatments can be counter-productive for growers who are trying to reduce the selection pressure for resistance by spraying less.

Most ATs, DTs and EILs developed for strawberry crops are based on assessment of thrips density in flowers, as there is a strong correlation between numbers of *F. occidentalis* per flower and fruit damage (Steiner & Goodwin, 2005b; Coll *et al.*, 2007a). Assessment of thrips in flowers gives an earlier warning of damage to young fruit than trap catches, although trap catches and sex ratio can also be useful indicators of damage (Steiner & Goodwin, 2005b). Trap catches are less reliable than flower sampling as they only catch adults and are more influenced by environmental conditions such as wind speed and temperature (Shipp & Zariffa, 1991). Published thresholds vary considerable, from 3 to 24 thrips per flower in strawberry (Table 4.1), partly as a result of the type of threshold defined (action, damage or economic) or thrips stages monitored (i.e. adults or adults and larvae combined). Other factors, such as cultivar (Rahman *et al.*, 2010), climate, time of year (Steiner & Goodwin, 2006) and sale price may also affect EILs (Coll *et al.*, 2007a). Predators are a key component for controlling pesticide-resistant *F. occidentalis* and damage thresholds can be relaxed in the presence of predators. Shakya *et al.* (2010) suggest that the presence of one *Orius* bug per flower can relax damage thresholds by about 40% and that the economic threshold can be increased by one or two thrips per flower in the presence or absence of pollen respectively, for every *N. cucumeris* per flower, based on their predation rates on strawberry (Shakya *et al.*, 2009). The amount of damage accepted by retailers and the fruit price changes with fruit availability through the season, which affects the pest tolerance. The variability in published data is such that further data are required to estimate EILs for thrips in UK strawberry.

To estimate thrips density in a crop, growers need a practical and effective method of sampling. Methods for assessing numbers of adult thrips in strawberry flowers, by eye,

were developed (see section 2.5, Chapter 2), as eye-counts are considered the most cost-effective sampling method for strawberry (García-Mari *et al.*, 1994) and other open-flowered crops, such as pepper (Shipp & Zariffa, 1991) and apple (Terry & DeGrandi-Hoffman, 1988). The sample size required to estimate a thrips population using visual assessment varies from 10 to 30 flowers in strawberry (García-Mari *et al.*, 1994; Laudonia *et al.*, 2000; Steiner & Goodwin, 2005a). Further data are required to identify the sample size required to estimate a thrips population in UK strawberry and to investigate the possibility of using time-efficient methods in UK strawberry.

The overall aim of this chapter was to identify EILs to help growers with decision-making for *F. occidentalis* management in semi-protected strawberry crops in the West Midlands area of the UK. Specific aims were to:

- (1) determine the relationship between the number of adult thrips per flower and subsequent fruit bronzing under UK conditions in order to estimate an EIL in the absence of predatory mites;
- (2) determine the effect of the predatory mite *Neoseiulus cucumeris* on fruit bronzing caused by *F. occidentalis* to estimate an EIL in the presence of predatory mites;
- (3) identify which strawberry fruit stage(s) are most susceptible to *F. occidentalis* damage and whether individual *F. occidentalis* adults or larvae cause more damage;
- (4) determine the distribution of adult and larval thrips on different strawberry flower and fruit stages in commercial crops, to see which fruit stages are exposed to the most thrips, which will in turn help to identify the timing of thrips damage;
- (5) measure the progression of flowers from bud to red fruit to help interpret the timing of sampling in relation to possible damage;
- (6) estimate the amount of bronzing that results in the down-grading of a strawberry fruit from class 1 (good quality fruit that is sold at the highest price) to class 2 (fruit that is reduced to a lower price due to bronzing), in a commercial pack-house;
- (7) validate the EILs in commercial UK strawberry fields in the West Midlands by comparing thrips density and fruit bronzing in different crops and seasons;
- (8) identify a cost-effective sampling method for growers.

4.2. Materials and Methods

For the rest of this chapter thrips refers to species in the Thripidae family unless specified otherwise. Counts of thrips in flowers in all experiments were carried out by eye using a $\times 7$ head lens (optiVISOR, LightCraft, London, UK), in medium-aged flowers (petals open, and with anthers starting to dehisce (Sampson & Kirk, 2012)). The flowers were picked and the petals peeled apart carefully so that the thrips could be seen. Eye counts were used because the results could then be related to grower monitoring which is done in the same way. All sampling was carried out between 10.00–15.00 h when thrips were most active and visible.

The economic injury level (EIL) in this study is defined as the lowest thrips population density that would cause economic damage, which is the amount of thrips bronzing (expressed as percentage of fruit surface bronzed) that would result in the down-grading of a strawberry fruit from class 1 (good quality fruit that is sold at the highest price) to class 2 (fruit that is reduced to a lower price due to bronzing). EILs were estimated using numbers of adult thrips per flower rather than motile stages (adults plus larvae). Both adults and motiles (adults and larvae) have been used to estimate thrips EILs (e.g. (Steiner & Goodwin, 2005a)), but adults were used in this study because eye-counts of larvae are known to be inaccurate (only 33% of larvae were found compared to alcohol counts) (González-Zamora & Garcia-Marí, 2003). Also, sampling different-aged flowers results in a greater variation of larval numbers compared to adults, which would increase the error. The field abundance of larval thrips in different-aged flowers, changed by a factor of seven between open and senescent flowers, compared to a factor of two for adult thrips (Sampson & Kirk, 2012). EILs have also been developed using thrips population estimates on fruit, but these were not considered in this study because they are less reliable (Steiner & Goodwin, 2005a).

There were insufficient data to include the cost and effectiveness of insecticide treatments within the EIL for UK strawberry, as insecticide resistance levels for individual farms were unknown and the efficacy of biopesticides was unproven in strawberry. Control costs could be factored in for different treatments at a later date, when efficacy data are available.

4.2.1. What is the effect of *F. occidentalis* on strawberry fruit damage?

To test the relationship between thrips density and subsequent fruit bronzing under UK conditions, different numbers of adult female thrips were caged on individual flowers and the amount of bronzing was recorded subsequently on the fruit that developed from those flowers. The experiment was carried out in an open-sided polytunnel (2 m × 5 m), at Keele University (N 53° 00.37' W 2° 27.71'), from late June to early August 2011. Strawberry (*Fragaria × ananassa*, cv. Camarillo) was grown in coir growbags (10 cm wide × 100 cm long, BVBSublime, Maasland, NL), each containing ten flowering and fruiting plants. Spontaneous outbreaks of aphids and spider mites were controlled with specific parasitoids and predators, *Aphidius* spp. (Hymenoptera: Aphidiidae) and *Phytoseiulus persimilis* (Acarina: Phytoseiidae) (Aphidsure and Phytosure respectively, BCP Certis, Ashford, UK). Powdery mildew was controlled by potassium bicarbonate sprays (1 g per 100 ml water). There were five treatments of 0, 2, 4, 8 or 16 adult female *F. occidentalis* per flower. The experiment was laid out in a randomised complete block design, with 17 blocks (each block was a separate growbag) and one replicate per block (one flower infested with each thrips density in each block). Five fully-open flowers, that were similar in size and position on the plant, were selected per block (growbag), then tagged and labelled using 50 mm sections of drinking straws, which were slit and placed over each flower onto the stems (Figure 4.3 A). Each flower was allocated a treatment using random numbers, and enclosed using a cage made of horticultural fleece (approx. 8 cm × 8 cm), tied to the stem with PTFE tape (polytetrafluoroethylene tape, 12 mm wide) (Figure 4.3. A). Thrips were taken from the culture at Keele University (see Chapter 2). Adult female thrips were obtained by tapping flowers over a white dish, then using a damp paintbrush to scoop them into Eppendorf tubes (12 mm × 37 mm). Mixed-aged females were used, as mixed-aged females would be present in the field, so it was a better representation of the field situation. Males were not tested as they do not produce larvae, which cause much of the damage. The use of females would therefore result in higher levels of damage than would be observed in field situations with mixed-sex populations. The proportion of adult males in flowers was typically about 28% (mean of adult thrips sampled through the 2011 season in semi-protected strawberry crops, n = 3,560 thrips). Tubes containing the appropriate numbers of thrips, or an empty tube for the control, were placed into the flower cages, opened, and left *in situ* to allow the thrips to move to the flower. Any invertebrates observed in the flowers were removed with a damp paintbrush

before the thrips were released. After one week the fleece covers were removed from the flowers to allow the fruit to develop normally. All fruit were harvested at the fully swollen white fruit stage (growth stage 85 (Meier *et al.*, 1994)), 30 days after they were infested, just as they were starting to turn pink. Each fruit was assessed for damage by counting the numbers of seeds surrounded by bronzing. The fruit were weighed and the total number of seeds per fruit was counted.

To test the relationship between fruit damage and thrips density, bronzing on white fruit (as above) was regressed on the numbers of thrips per flower. An EIL was calculated from the regression equation, as the number of adult thrips per flower that corresponded with bronzing on white fruit over 10% of the fruit surface area (about 29 seeds in this experiment), which would result in fruit down-grading (from class 1 to class 2) in a commercial pack-house (see 4.3.4).

4.2.2. What is the effect of *N. cucumeris* on *F. occidentalis* fruit damage?

To test the effect of *N. cucumeris* on fruit bronzing caused by thrips, adult female *F. occidentalis* were caged on individual flowers at a density of either four (4.2.2.1) or eight (4.2.2.2) per flower, with or without the predator *N. cucumeris*, and the amount of bronzing was recorded subsequently on the fruit that developed from those flowers. If reduction in damage exceeded 50%, this would also provide supporting evidence that larvae are responsible for most (i.e. >50%) of the damage to strawberry. *Neoseiulus cucumeris* feed mostly on first instar thrips larvae because they are less able to catch larger prey (Bakker & Sabelis, 1989). The experiment was carried out at a thrips density of four and eight per flower, as these were around the lower and upper EILs observed in the field (see section 4.3.5). Both experiments were carried out in the same polytunnel (at Keele University), using the same growing system, strawberry cultivar (cv. Camarillo) and cultural methods described in section 4.2.1.

4.2.2.1. At four adult thrips per flower

The two treatments were with and without *N. cucumeris*. The experiment was laid out in a randomised block design, with 4 blocks, on 11 June 2013. Each block consisted of two separate growbags and two replicates per treatment (two flowers with predators in one growbag and two flowers without predators in a second growbag). The treatments were in separate growbags and each growbag was surrounded by blue sticky traps placed on the

ground to reduce the spread of predatory mites from treated to untreated growbags (Figure 4.3.C). Newly-opened flowers of a similar size were selected, and then each was infested with 4 adult female *F. occidentalis*, and enclosed in a nylon pyramid teabag cage (75 mm long × 33 mm wide at the top, Tea Forte, St Albans, UK, Figure 4.3. B), which was sealed with clear adhesive tape. Treated flowers were infested with 5 active *N. cucumeris* (Ambsure (c), BCP Certis, Ashford, UK), which is equivalent to a good establishment of the predatory mites in the field (Rahman *et al.*, 2012). Mixed-aged and mixed-sex *N. cucumeris* were used to match releases made by growers. The predatory mites were collected directly from the commercial package (Ambsure (c)) using a damp paint brush, and transferred into Eppendorf tubes (12 mm × 37 mm). Tubes containing the predators, or an empty tube for the control, were placed into the flower cages, opened, and left *in situ* to allow the mites to move to the flower. After one week, the cages were removed to allow the fruit to develop normally. All fruit were harvested at the fully swollen white fruit stage as they were starting to turn pink, 27 days after they were infested. The fruit were harvested at this stage because some fruit were ripening faster than others, so harvesting early allowed direct comparison of all fruit at the same colour stage on the same date. Also, a toad and a blackbird were feeding on fruit as they ripened, so the fruit were harvested in case they got eaten! Each fruit was assessed for damage by counting the number of seeds surrounded by bronzing. The fruit were weighed and the total number of seeds per fruit was counted.

4.2.2.2. At eight adult thrips per flower

The experiment was carried out as above (4.2.2.1), except with eight adult female *F. occidentalis* per flower instead of four. The experiment was set up on 4 June 2013 and harvested at the white fruit stage, after 30 days.

4.2.2.3. Adjustment of the Economic Injury Level (EIL) in the presence of *N. cucumeris*.

The reduction in fruit damage observed in the presence of *N. cucumeris* from the experiments above (see 4.2.2.1, 4.2.2.2) was used to calculate the mean reduction of damage (number of seeds surrounded by bronzing) per individual *N. cucumeris* per flower. The comparisons would give an indication as to how the EIL could be relaxed when predatory mites are present.

4.2.3. When does fruit damage occur during fruit development?

The susceptibility of different flower or fruit stages to adult and larval thrips damage was tested under controlled conditions. By combining this information with the distribution of thrips between different strawberry stages in the field, the timing of damage can be estimated. This in turn will help to interpret EILs and prediction of damage.

4.2.3.1. Which flower or fruit stages are susceptible to adult or larval thrips damage?

To determine the relative susceptibility of different flower and fruit stages to adult or larval thrips, the same numbers of adult or larval thrips were caged on different strawberry flower or fruit stages for seven days, then thrips were regularly removed from the fruit thereafter and the amount of bronzing on the fruit that developed subsequently was scored, compared to an untreated control. The experiment was carried out in August 2012, in the Keele polytunnel, using the same growing system, strawberry cultivar (cv. Camarillo) and cultural methods described in 4.2.1. The experiment was laid out in a randomised complete block design, with 10 blocks (each block was a separate growbag) and one replicate per treatment ($n = 10$ flowers or fruit). There were 12 treatments; four stages of strawberry (mid-aged open flowers, green fruit, white fruit and red fruit) infested with six *F. occidentalis* second instar larvae (mixed sex), six adult female *F. occidentalis* or no thrips (control). Each flower or fruit was allocated a treatment using random numbers, and enclosed using a cage made of horticultural fleece (approx. 8 cm \times 8 cm), tied to the stem with PTFE tape (polytetrafluoroethylene tape, 12 mm wide). Thrips were sourced from the culture at Keele University (see Chapter 2) using the methods described in 4.2.1. Any invertebrates observed in the flowers were removed with a damp paint brush before the thrips were released. After one week the fleece covers and all visible thrips were removed with a damp paintbrush and then every three days until harvest. All fruit were harvested when they reached the red fruit stage so that damage could be compared on the same fruit colour. Fruit damage was assessed by counting the number of seeds surrounded by bronzing. The amount of damage caused by thrips on the different fruit or flower stages and the amount of damage caused by adult or larval thrips were compared. Eight fruit had been partially eaten (possibly by a toad) and these were excluded from the analysis.

4.2.3.2. Within-plant distribution of thrips

The distribution of thrips was assessed on different strawberry plant parts in four semi-protected strawberry fields (cv. Camarillo) to determine which fruit stages had the greatest exposure to thrips. When combined with information on the relative susceptibility of the different fruit stages, this would indicate when most of the damage is occurring, which could help growers to time treatments to prevent damage. As the distribution could vary with thrips density, it was tested in two fields with high thrips densities (>10 adult thrips per flower) and in two fields with low thrips densities (<5 adult thrips per flower). Samples were taken on 5 July 2011 (field 3, Table 2.1), 10 August 2012 (field 7, Table 2.1), 22 August 2012 (field 4, Table 2.1) and 13 September 2012 (field 4, Table 2.1). On each occasion, ten plants were selected at random from a 10 m section of bed. One fully expanded leaf, one open flower, one senescent flower, one button fruit, one green fruit, one white fruit and one red fruit were selected from each plant and placed separately into pots of 70% alcohol ($n = 10$ for each plant part per site). In the laboratory, thrips were washed off the samples and the numbers of adult and larval thrips were counted. The proportion of adults or larvae on each plant part was calculated as a percentage for the fields with high and low thrips numbers separately. To test whether thrips density affected the distribution on plants, the proportion of the thrips population on red and white fruit in two fields with low thrips densities and two fields with high thrips densities were compared.

4.2.3.3. Flower progression in relation to *F. occidentalis* life cycle

To help interpret the distribution of thrips on the different plant parts, six flower buds (unopened) were selected at random on 29 June 2011, tagged and their progression recorded daily until harvest (Keele polytunnel, cv. Camarillo). Temperatures were recorded as described in Chapter 2. The duration of each stage was calculated, then compared with published data on the time taken for different development stages of *F. occidentalis* on strawberry at the temperatures recorded (Nondillo *et al.*, 2008).

4.2.4. Estimation of the EIL in a commercial pack-house

Fruit in pack-houses are graded into class 1 (good quality fruit that is sold at the highest prices), class 2 (fruit that has a small amount of damage and is sold at a lower price) and waste (fruit of poor quality that cannot be sold). An EIL was defined as the amount of damage on red fruit, above which fruit is downgraded from class 1 to class 2.

This was determined by comparing the amount of bronzing on 25 higher priced fruit (class 1 fruit) and 25 fruit that had been downgraded to a lower price (class 2 fruit), using harvested red fruit that had been sorted in a pack-house. This was done on 13 September 2012 on cv. Finesse and on 2 October 2012 on cv. Camarillo. While different cultivars may be more or less susceptible to thrips damage, they were graded on the same criteria. Fruit was graded and selected by pack-house staff, individual fruit were then removed that had been downgraded for reasons other than bronzing, such as size, shape, bruising and disease. Bronzing was quantified on the remaining fruit by counting the numbers of seeds surrounded by bronzing per fruit in the laboratory using a $\times 7$ head lens (optiVISOR). To give an indication of where the EIL between class 1 and class 2 fruit lay, the inter-quartile ranges of bronzing for both classes of fruit were compared and the threshold was selected from between the lower quartile of class 2 fruit and upper quartile of class 1 fruit, such that the majority of class 2 fruit was above and the majority of class 1 fruit was below.

The damage on fruit from the pack house was assessed on red fruit whereas the field data were obtained from white fruit, where damage shows up more easily. The assessment of white fruit enabled comparison of the same fruit stage between fields and dates, as red fruit of comparable ripeness was not always available following picking, and selective picking of undamaged red fruit would have biased the red fruit samples. The assessment of bronzing on red fruit from the pack-house and white fruit from the field was considered broadly similar because the red fruit assessments were done under a bright light with magnification so that the bronzing showed up well, so a 1:1 conversion was used. A more precise conversion was used for estimating economic crop loss due to bronzing in Chapter 6. Because damage shows up more easily on white fruit, the resulting thresholds on white fruit may be considered conservative.

4.2.5. Validation of EILs in commercial crops

Previous controlled damage experiments showed that five or more adult thrips per flower resulted in fruit bronzing that would result in downgrading in a commercial pack-house (10% of the fruit surface bronzed, section 4.3.2) but that eight adult thrips per flower could be tolerated when there was good establishment of *N. cucumeris* (section 4.3.2). The aim of this study was to test the relationship between thrips density and fruit damage in commercial crops, by assessing the numbers of adult thrips per flower and amount of fruit bronzing weekly in two semi-protected crops (cv. Camarillo) through the growing season

in 2011 and on single occasions in seven semi-protected strawberry crops of varying cultivars and thrips densities in 2012 and 2013.

4.2.5.1. What is the relationship between flowering periods, thrips per flower and fruit damage through the season?

Phenological data were collected weekly from two crops (cv. Camarillo) on separate farms, from first flowering to final harvest, in 2011, as described in Chapter 3, section 3.2.1 (Figures 3.1, 3.2). The cultivar Camarillo was selected to allow direct comparison with the controlled damage experiments above. In addition to the data collected on flowers per plant, thrips per flower and thrips per trap (described in Chapter 3), fruit were assessed for bronzing damage at the same time and on the same plants. Once fruit were present, one fully-expanded white fruit was selected from each sample plant. If none were present on the sample plants, the nearest fruit was sampled. On each date, fruit bronzing was assessed by eye, using a $\times 7$ head lens (optiVISOR) and recorded using a five-point scale:

- 0: Absent (i.e. free from thrips damage).
- 1: 'calyx' - tracking under calyx but not on fruit.
- 2: 'slight' - bronzing on the fruit (one patch of russetting around 1-3 seeds).
- 3: 'moderate' - bronzing (from 4 seeds to 50% of the fruit surface area damaged).
- 4: 'severe' - more than 50% of the fruit surface area damaged.

The five-point damage scale was used only in the first year of the project before the EILs had been determined and would need to be revised to take into account the 10% EIL (see 4.3.4) if used again. Absolute damage assessments (numbers of seeds surrounded by bronzing) were used in years 2 and 3 of the study because it gave a more precise linear scale.

The presence or absence of *Neoseiulus* spp. was recorded per fruit. Twenty *Neoseiulus* spp. individuals were selected at random from field 3, mounted on slides, using methods for mounting thrips described in Chapter 2, and identified to species under a compound microscope (Leica).

To indicate which length of time between thrips density in flowers and fruit damage gives the best correlation, regression analyses of bronzing on white fruit on thrips density (measured as above) was carried out with time lags between flower counts and fruit damage of between zero and six weeks. First flower bud to red fruit takes about six weeks

under UK conditions (see 4.3.3.3). Knowing the time of damage in relation to thrips density in flowers would help growers to decide how frequently to sample and how quickly they need to act to prevent further damage.

To test whether thrips per flower or trap catch was a better predictor of damage, the relationship between fruit bronzing and thrips density was quantified by regressions of bronzing on white fruit (from the scale above) on numbers of adult thrips per flower or numbers of thrips per pheromone trap.

4.2.5.2. *EILs observed in commercial crops.*

To test the relationship between thrips density in flowers and fruit damage in a wider range of crops, bronzing on white fruit (recorded using the scale above in 2011 or as the numbers of seeds surrounded by bronzing on white fruit in 2012 and 2013) was regressed on the mean numbers of adult thrips per flower in different fields, cultivars and years. The data were taken from different experiments carried out throughout the study and the methods for collecting the data will not be repeated here. The experiment and field from which each data set came is referenced in the results table (Table 4.4). The predatory mite *N. cucumeris* was released in all the fields sampled, but by varying amounts and frequencies. In addition to the fruit damage assessments, each fruit was picked and examined by eye, using a $\times 7$ head lens (optiVISOR) and the presence or absence of *Neoseiulus* spp. was recorded per fruit. Analysis was carried out on data collected weekly through the growing season from two crops in 2011 (see 4.2.5.1), but on single dates in the other crops. Thrips density on a particular date was compared to white fruit bronzing on the same date, as this gave the best correlation (see 4.3.5.1) and is convenient for growers to use. The thresholds were calculated from the regression equations, as the numbers of adult thrips per flower that corresponded with bronzing on white fruit over 10% of the fruit surface area (about 30 seeds) and resulted in fruit down-grading (from class 1 to class 2) in a commercial pack-house (see 4.3.4).

4.2.6. **How many flowers should be sampled to estimate thrips density?**

To determine the number of flower samples required to estimate thrips populations, the distribution of thrips was compared to thrips density in three crops (cv. Camarillo), monthly from mid-April to mid-August 2012. Camarillo crops (fields 3, 4, 10, Table 2.1) were used so that the results could be related to the EIL experiments, which were estimated

for the same cultivar. In each field there were eight sub-plots (one tunnel wide x 15 m) evenly spread through the field, but at least 20 m in from the edge to reduce edge effects. On each assessment date, 40 medium-aged flowers were sampled regularly from across each plot and the numbers of adult thrips per flower were counted by eye, using a $\times 7$ head lens (optiVISOR), as above. In total, 1920 Camarillo flowers were sampled in crops with different thrips densities through the season.

Aggregation indices were calculated using Taylor's power law (Taylor, 1961) which has been used to describe thrips populations in strawberry (Laudonia *et al.*, 2000; Steiner & Goodwin, 2005a) and other crops (Navarro-Campos *et al.*, 2012) and the same methods were used to enable direct comparison. Taylor's power law shows that mean (m) and variance (s^2) increase together according to the equation $s^2 = a m^b$, where s^2 is the sample variance, m is the sample mean, a is sampling constant relating to sample size and b is Taylor's index of aggregation, which is characteristic of the species. The constants a and b were estimated using a regression after log-log transformation ($\log s^2 = \log a + b \log m$). An aggregation index above 1 indicates clumped populations. Taylor's constants (a and b) were then used to calculate the minimum sample size (n) required to estimate thrips density with precision levels of 80% and 90%, using $n = a m^{b-2} / D^2$ (Green, 1970), where $D = 0.2$ for 80% accuracy or 0.1 for 90% accuracy. A population density estimate with a SEM of 25% is considered accurate enough for damage assessment studies (Southwood, 1978).

The possibility of reducing sampling time by using binomial sampling (presence or absence) was investigated by comparing the mean number of thrips per flower with percentage flowers infested (Ugine *et al.*, 2011). The mean value (m) was adjusted using the equation $m' = m^{1-0.5b}$ (Southwood, 1978) to give a linear relationship when m' is plotted against percentage flowers occupied, where b is Taylor's index of aggregation (see above).

4.2.7. An action plan for growers

An action plan was drafted showing how the EILs (with and without predatory mites) devised from experimental and field data might be used to improve integrated pest management of thrips in strawberry.

4.2.8. Statistical analysis

General statistical methods are described in Chapter 2 (section 2.8). Further details specific to sampling are shown in 4.2.6.

4.3. Results

4.3.1. What is the effect of *F. occidentalis* on strawberry fruit damage?

When thrips were confined on flowers, fruit bronzing increased with the number of adult female *F. occidentalis* per flower (one-factor ANOVA, $F_{(4, 63)} = 51.9$, $P < 0.001$, Figure 4.4 A). At low thrips densities (two per flower) there was slight brown tracking under the calyx, with about 8% of the white fruit surface bronzed. At medium thrips density (four to eight per flower) bronzing covered 18 to 27% of the white fruit surface. At high thrips density (16 per flower) about 80% of the white fruit surface was bronzed. Fruit weight averaged 7.2 ± 0.5 g with no significant difference in weight observed between treatments (one-factor ANOVA, $F_{(4, 78)} = 0.87$, $P = 0.48$, Figure 4.4 B). Mean number of seeds per fruit \pm SEM was 291 ± 15 seeds. Minimum and maximum temperature and humidity was 6°C: 30°C and 49%: 96% RH respectively.

A log (n+1) transformed regression of fruit damage on thrips density gave an EIL of 4.6 adult thrips per flower to give fruit bronzing over $10 \pm 0.4\%$ of the fruit surface in the absence of predators: $\log \text{bronzing (number of seeds + 1)} = 0.31 + 1.56 \log (\text{thrips} + 1)$; $F_{(1,82)} = 144$, $P < 0.001$; $R^2 = 64\%$).

4.3.2. What is the effect of *N. cucumeris* on *F. occidentalis* fruit damage?

4.3.2.1. At four adult thrips per flower

The addition of *N. cucumeris* (5 per flower) to flowers containing four adult female *F. occidentalis* reduced fruit bronzing from about 26% to $<1\%$ of the fruit surface (one-factor ANOVA with blocks, $F_{(1, 3)} = 11.6$, $P = 0.042$, Figure 4.5. A), bringing bronzing below the EIL (section 4.3.4), to a level where there was little detectable damage, so little further reduction was possible. There was no significant difference observed between treatments in fruit weight (one-factor ANOVA with blocks, $F_{(1, 3)} = 0.02$, $P = 0.90$, Figure 4.5. B), or number of seeds per fruit (one-factor ANOVA with blocks, $F_{(1, 3)} = 7.46$, $P = 0.07$), which averaged (\pm SEM) 314 ± 17 and 351 ± 22 with and without *N. cucumeris* respectively. At the end of the experiment there were 3.8 ± 1.0 predators per fruit in treated plots and 1.0 ± 0.5 predators per fruit in untreated plots (one-factor ANOVA with blocks, $F_{(1, 3)} = 11.0$, $P = 0.045$), indicating that there had been some movement of predators from control to treated growbags over six weeks. Minimum and maximum temperatures and humidities were 4.5°C, 30°C and 48%, 95.5% RH respectively.

4.3.2.2. At eight adult thrips per flower

The addition of *N. cucumeris* (five per flower) to flowers containing eight adult female *F. occidentalis* reduced fruit bronzing from about 51% to about 4% of the fruit surface (one-factor ANOVA with blocks, $F_{(1,3)} = 75.5$, $P = 0.003$, Figure 4.6. A), bringing bronzing below the EIL (see 4.3.4). There was no significant difference observed between treatments in fruit weight (one-factor ANOVA with blocks, $F_{(1,3)} = 0.05$, $P = 0.84$, Figure 4.4. B), or number of seeds per fruit (one-factor ANOVA with blocks, $F_{(1,3)} = 0.6$, $P = 0.49$), which averaged (\pm SEM) 353 ± 9 and 361 ± 7 with and without *N. cucumeris* respectively. At the end of the experiment there were 3.5 ± 0.2 predators per fruit in treated plots and 0.5 ± 0.2 predators per fruit in control plots (one-factor ANOVA with blocks, $F_{(1,3)} = 87.4$, $P = 0.003$), indicating that there had been some movement of predators from treated to control growbags over six weeks. Minimum and maximum temperatures and humidities were 4.5°C, 30°C and 48%, 95.5% RH respectively.

4.3.2.3. Adjustment of the EIL in the presence of *N. cucumeris*.

In the two controlled experiments above (see 4.3.2.1, 4.3.2.2), each adult thrips per flower resulted in bronzing around 21 ± 2 seeds per fruit in the absence of predatory mites. The estimated bronzing from 4.6 adult thrips (the EIL calculated in the absence of predatory mites in 4.3.1) is therefore 97 ± 9 seeds (21×4.6). At four thrips per flower, five predatory mites per flower prevented nearly all the thrips damage (Figure 4.5 A). At eight thrips per flower, five predatory mites per flower reduced the damage by 171 ± 27 seeds (Figure 4.6 A), which is equivalent to a reduction of 34 ± 5 seeds per mite per flower. As each mite reduced bronzing by 34 seeds, then a higher thrips level can be tolerated that would cause more damage without mites. Based on these results, the EIL would increase from 4.6 adult thrips per flower to 6.2 adult thrips per flower where one predatory mite is present, according to the calculation $((97+34)/97) \times 4.6$. Therefore the EIL could be relaxed by 1.6 adult thrips per flower for each *N. cucumeris* adult present. This is an approximation that should be interpreted with some care as predation rates and relative damage are likely to vary with thrips and predator density. Table 4.5 shows how this might be used by growers for decision-making.

4.3.3. When does damage occur during fruit development?

4.3.3.1. Which flower or fruit stages are susceptible to adult or larval thrips damage?

Both adult and larval *F. occidentalis* caused damage to all stages of strawberry tested (Figure 4.7). There was a trend towards more damage when flowers were infested with thrips compared to fruit stages, although the overall differences were not statistically significant (two-factor ANOVA, $F_{(3,100)} = 1.5$, $P = 0.23$) and there was no interaction between stage \times treatment (two-factor ANOVA, $F_{(6,100)} = 0.5$, $P = 0.78$). The trend towards more damage when thrips were released earlier might result from extra damage caused by thrips that hatched between removal times (every three days), which would accumulate over time on plant parts that were infested earlier. There was a significant difference between the amount of damage between thrips larva, thrips adult and control treatments (two-factor ANOVA, $F_{(2,100)} = 77.6$, $P < 0.001$). Tukey's test showed that larvae caused nearly twice ($\times 1.7$) as much damage as adults over a week and both stages caused more damage than controls, with mean bronzing (numbers of seeds) \pm SEM being 15.0 ± 2.6 , 8.9 ± 1.4 and 0.9 ± 0.2 for six larvae, six adults and untreated plant parts respectively. The amount of damage and timing of damage therefore relates to the numbers of larval and adult thrips present on the different stages of fruit development. The distribution of thrips was tested in commercial crops with low and high thrips numbers (see next section).

4.3.3.2. Within-plant distribution of thrips

Adult thrips were concentrated in strawberry flowers (74% and 79% in flowers in fields with low and high thrips density respectively), with the rest spread between fruit of different stages of ripeness and relatively few ($<1\%$) on the leaves (low thrips, two-factor ANOVA, $F_{(6,6)} = 15.5$, $P = 0.002$, Figure 4.8 A; high thrips, two-factor ANOVA, $F_{(6,6)} = 34.3$, $P < 0.001$, Figure 4.8 B). There was no significant difference between the proportion of adults on later stage fruit (red and white fruit) between the fields with low thrips density or high thrips density (one-factor ANOVA, $F_{(1,2)} = 0.03$, $P = 0.87$).

Larval thrips were most numerous in the senescent flower and green fruit stages in the fields with low thrips densities, then numbers of larvae declined as the fruit developed with about 6% on the red and white fruit stages (combined) and relatively few ($<1\%$) on the leaves (two-factor ANOVA, $F_{(6,6)} = 14.4$, $P = 0.002$, Figure 4.8 C). In fields with high thrips densities, the larvae were more evenly distributed between the different fruit stages

with 29% on the red and white fruit stages (combined) (two-factor ANOVA, $F_{(6, 6)} = 5.0$, $P = 0.035$, Figure 4.8. D). There were proportionally more larvae on later stage fruit (red and white stage fruit) in fields with high thrips density than in fields with low thrips density (one-factor ANOVA, $F_{(1,2)} = 725.3$, $P < 0.001$).

As damage can occur at all stages of fruit development (see 4.3.2.1), the results suggest that most damage would occur in the late flowering and green fruit stages in fields with low thrips densities, whereas damage is likely to occur more evenly throughout fruit development in fields with higher thrips densities.

4.3.3.3. Flower progression in relation to *F. occidentalis* life cycle

The progression of strawberry flowers from first opening of buds to harvest took approximately six weeks (42 days) from the end of June to mid-August (Table 4.2). Mean temperature was 15.7°C during this time, which was relatively cool for the time of year. In published data on *F. occidentalis* on strawberry at a constant 16°C, the life cycle (egg to adult) took 33 days, with eggs taking about 9.8 days, larval instars taking 14.4 days and pupae taking 8.9 days (Nondillo *et al.*, 2008). The relative duration of the different thrips life stages (using data from Nondillo *et al.* (2008)) in relation to the different flower and fruit stages from Table 4.2 is illustrated in Figure 4.9. When adult thrips occur in the open flower stage (over 70% of adult thrips were found in flowers in field populations, see 4.3.3.2), the larvae hatching from eggs laid by those adults would occur mainly in the green fruit stages and would start to drop off to pupate as the fruit turned white, resulting in a drop in larval numbers at the white fruit stage, which is observed in fields with low thrips populations (Figure 4.8 C). In this situation a second generation of thrips adults would start to hatch near the end of fruit development but would not necessarily return to the same fruit. First instar larvae are found in very young buds (see Chapter 2), as might occur when eggs are laid in the sepals before the flowers open or if the larvae move into young buds and flowers from other plant parts in search of pollen. In this situation, most larvae would have pupated whilst the fruit was still green but a second generation of larvae would occur when the fruit are in the white fruit stage, although these would not necessarily be on the same fruit. The overlapping generations present in flowers can result in larvae being present in similar numbers throughout fruit development, as was observed in fields with high thrips numbers (Figure 4.8 D). When a thrips population is increasing, proportionally

more damage would occur in the later stages of fruit development, reflecting the higher thrips density at the time.

4.3.4. Estimation of the EIL in a commercial pack-house

Harvested red fruit that had been downgraded to class 2 in the pack-house showed significantly more bronzing than class 1 fruit (one-factor ANOVA, $F_{(1,45)} = 51.4$, $P < 0.001$, cv. Camarillo; $F_{(1,48)} = 32.5$, $P < 0.001$, cv. Finesse). In pack-house fruit, 50% of class 1 fruit had bronzing surrounding 5-20 (cv. Camarillo) and 2-32 (cv. Finesse) seeds and 50% of class 2 fruit had bronzing surrounding 34-93 (cv. Camarillo) and 30-80 (cv. Finesse) seeds (Figure 4.10), pointing to an EIL in the region of 30 for bronzing around seeds per red fruit, which was about 10% of the fruit surface bronzed. This was considered a reasonable estimate as >80% of class 1 red fruit had bronzing below this threshold and >80% of class 2 red fruit had bronzing above this threshold in both varieties assessed.

4.3.5. Validation of EILs in commercial crops

Whilst mixed thrips species were present in all fields, the majority of thrips were *F. occidentalis* (typically >90%) at times when most fruit bronzing was observed, from late-July through to September (see Chapter 3, Figure 3.4).

4.3.5.1. What is the relationship between flowering periods, thrips per flower and fruit damage through the season?

When two crops were sampled weekly through the season in 2011, a small amount of fruit damage was observed in early-June from autumn-initiated flowers, but fruit damage increased rapidly from mid-July, which corresponded with a similar increase in numbers of adult thrips per flower (Figure 4.11). In both crops, thrips density in flowers increased at the end of flower flushes, when thrips concentrated into fewer flowers, resulting in increased numbers of thrips per flower and subsequent fruit damage (Figure 4.11). Flower density in crops could be a key factor in predicting the timing of thrips damage.

Grower records in 2009-2011 showed a small amount of class 2 fruit (5% fruit downgraded) in late-May to early-June (week numbers 23-25), which corresponded with the end of the first flower-flushes in second-year crops, and a significant amount of class 2 fruit (15-30% fruit downgraded in the worst affected fields) from late July through August

(week numbers 30-36), which corresponded with the main period of thrips activity (S. Clarke, pers. comm, 2011).

Regression analysis of white fruit damage on thrips density in flowers showed the best correlation when both measurements were obtained on the same date, but became progressively weaker and the regression slopes less steep as the time gap increased (Table 4.3). This indicates that most fruit damage was occurring relatively recently (e.g. in the previous 10 days). The correlations between thrips density in flowers and fruit damage one and two weeks later were still statistically significant and reasonably strong ($R^2 = 69\%$ and 70% in fields 2 and 3 respectively), so the assessment of thrips density in flowers is a good indicator of fruit damage at the same time or soon after (e.g. up to two weeks after flowering), but not reliably beyond that (Table 4.3). Only one plot was used for the analysis for field 2 as there were insufficient thrips in the other plot to cause fruit damage and early season data were excluded because no thrips were recorded until both fruit and flowers were present. This explains why the number of data points decreases with the lag in field 2. In field 3, thrips were present from the start of sampling and there was six weeks of flowering before fruit was present, which explains why the number of data points remains the same for the different lags.

The relationship between the mean numbers of adult thrips per flower and mean fruit bronzing on the same dates through the season was similar in the two commercial crops:

- field 2: $\log(\text{bronzing} + 1) = 0.01 + 0.63 \log(\text{thrips density} + 1)$, $F_{(1,12)} = 101.7$, $P < 0.001$, $R^2 = 89\%$ and
- field 3: $\log(\text{bronzing} + 1) = 0.02 + 0.62 \log(\text{thrips density} + 1)$, $F_{(1,34)} = 108.4$, $P < 0.001$, $R^2 = 75\%$,

where, bronzing = the numbers of seeds on white fruit surrounded by bronzing and thrips density = the numbers of adult thrips per flower.

The use of the damage scale did not give a very precise measure for defining an EIL, but 10% fruit surface bronzing equated to just above two on the scale used. Fruit damage reached this threshold when there were 5.0 ± 0.3 per flower in both fields, where predatory mite establishment was relatively low. The adult thrips population was higher in field 3 than in field 2 at the start of the season, but there were proportionally fewer thrips larvae (compared to adults) in field 3, where 42% of the fruit were infested with predatory mites compared to 4% in field 2 (Figure 4.11). A higher EIL might be expected in field 3, where

there were more predatory mites, but the use of a damage scale did not allow for sufficient differentiation in this case. 95% of the *Neoseiulus* spp. collected from field 3 were identified as *N. cucumeris* and 5% were *N. californicus* (n = 20 mites).

The use of trap catch rather than counts of thrips in flowers resulted in a significant but weaker correlation with fruit damage in the same fields and on the same dates through the season:

- field 2: $\log(\text{bronzing} + 1) = -0.44 + 0.36 \log(\text{thrips per trap} + 1)$, $F_{(1,15)} = 82.7$, $P < 0.001$, $R^2 = 62\%$ and
- field 3: $\log(\text{bronzing} + 1) = -0.19 + 0.22 \log(\text{thrips per trap} + 1)$, $F_{(1,28)} = 17.5$, $P < 0.001$, $R^2 = 36\%$,

indicating that flower sampling gives a better measure of thrips population density than trap catch for the prediction of fruit damage.

4.3.5.2. EILs observed in commercial crops.

EILs of between 5 and 11 adult thrips per flower were identified in six commercial crops where thrips damage had resulted in some downgrading of fruit to a lower price (Table 4.4). No economic crop loss due to thrips damage was observed in three crops where thrips numbers remained below 5 adults per flower throughout the season (Table 4.4).

The lowest EIL (economic fruit damage when there were 5 adult thrips per flower) was observed in the field with the lowest cover of predatory mites and the highest EIL (economic fruit damage when there were 11 adult thrips per flower) was observed in the field with the highest cover of predatory mites (Table 4.4). These data broadly support the EILs observed in controlled experiments with and without *N. cucumeris* (see 4.3.1, 4.3.2), but cannot be quantified further as the numbers of predatory mites per fruit were not counted nor were they routinely identified to species. The data should be viewed with some caution as the establishment of predatory mites varied between fields, within field and between sample dates.

4.3.6. How many flowers should be sampled to estimate thrips density?

The relationship between mean numbers of adult thrips per flower and variance for the Camarillo crops sampled estimated Taylor's coefficients $a = 0.12 \pm 0.01$ and $b = 1.12 \pm$

0.02 (log variance = $0.11 + 1.12 \log \text{mean}$, $P < 0.001$, $R^2 = 97\%$). A slightly aggregated population is indicated by the aggregation index b , which is just >1 . Taylor's coefficients were used to calculate the minimum sample size required to estimate thrips density in flowers at 80% and 90% accuracy (Figure 4.12 A). For growers, who are monitoring to determine EILs, the sampling of 8 and 32 flowers from an area would be sufficient to estimate the population with 80% and 90% accuracy respectively when the mean thrips density is 5 adult thrips per flower and 4 and 17 flowers when the mean thrips density is 11 adult thrips per flower (these being the thrips densities for lower and upper EILs estimated without and with predatory mites). The sampling of 10 flowers per area of interest would be sufficient to give $>80\%$ accuracy in estimating EILs of 5 to 11 adult thrips per flower.

The possibility of reducing sampling time by using binomial (presence, absence) sampling was tested by comparing mean thrips per flower with the percent of flowers infested (Figure 4.12 B). When the mean was adjusted to a linear relationship ($m' = m^{1-0.5b}$), the percentage of flowers infested = $-8.07 + 59.6 \times m'$. Thrips reached 100% flower occupation when the mean thrips per flower was about 4 thrips per flower (3 and 4 adult thrips per flower equate to 88% and 101% flower occupancy respectively). This could be a useful as a rough guide for growers as an alert that the EIL (about 5 adult thrips per flower) is approaching and that more detailed monitoring is needed. If valid, then presence and absence sampling could be used until 100% occupancy while thrips numbers are still below damaging densities, then growers could change to counting numbers of adult thrips per flower.

4.3.7. An action plan for growers

An action plan for thrips management on strawberry (cv. Camarillo) in the West Midlands, UK, is shown in Table 4.5. The table suggests possible actions according to thrips density and establishment of predatory mites, according to EILs devised from experimental and field data. Further testing in commercial crops is required to validate the thresholds and actions in different cultivars and regions of the UK before it could be recommended to growers.

4.4. Discussion

The pattern of bronzing observed on strawberry fruit caused by thrips in this study was consistent with that found in Australia (Steiner & Goodwin, 2005a) and elsewhere, with a netting pattern around the seeds when damage occurred in the early stages of fruit development (Figure 4.1 B) and damage in the pit surrounding the seeds when it occurred on swollen fruit (Figure 4.1 C, D). Severely damaged fruit had a dull, seedy appearance (Figure 4.1 E). The position of damage on the fruit therefore gives a clue to when most of the damage occurred during fruit development. Thrips damage did not explain all the bronzing, and bronzing associated with pesticide treatment (e.g. Figure 6.10 D, Chapter 6), physical damage or high temperature was observed in the field. Thrips damage did not affect fruit weight in the controlled experiments in this study, which contrasts with other work (Coll *et al.*, 2007a). This may be because lower numbers of thrips per flower were tested in this study to reflect typical field populations (2-16 adult thrips per flower), compared to 60 adult thrips per flower used by Coll *et al.* (2007a). Waste fruit affected by thrips damage in the commercial fields in the UK appeared to be small (Figure 4.1 E), but this was not quantified and fruit were down-graded as a result of bronzing before weight was affected. In line with recent studies (e.g. Nondillo *et al.* (2010)), no fruit deformation was observed as a result of thrips damage.

To make timely control treatments, growers need to know when the majority of damage is occurring during the approximately six week period (in the UK) that it takes from bud-break to harvested red fruit. Coll *et al.* (2007a) found the ripe fruit most susceptible to thrips bronzing and Steiner and Goodwin (2005a) suggested that the most important damage was done at the young green fruit stage. In contrast, all stages of fruit and late flowering (cv. Camarillo) were found susceptible to thrips bronzing damage in this study, as found by Nondillo *et al.* (2010), suggesting that damage could occur at any time from flowering to harvest. The data from Coll, *et al.* (2007a) may reflect the cultivar used (cv. 328, Tamar) which apparently tolerated higher numbers of thrips without bronzing damage than other cultivars in comparable studies (Table 4.1). If all stages of fruit are equally susceptible, the predicted timing of damage reflects the numbers of thrips present at each fruit development stage. Second-instar *F. occidentalis* larvae caused proportionally more damage per individual than adults (Figure 4.7) and larval *F. occidentalis* have been found to cause more damage than adults in other crops (Pearsall, 2000). Although

differences in damage caused by adults and larvae were statistically significant in my study, the error was large (Figure 4.7), which could have resulted from adults escaping from the cages or failure to remove all the thrips after the plants had been infested for a week. Further controlled experiments comparing damage caused by adult and larval thrips on different stages of fruit, over a short period of time (e.g. a day) would provide useful back-up information. The large reduction in damage (>90%) observed in the presence of *N. cucumeris*, which only feed on thrips larvae, provided supporting evidence that *F. occidentalis* larvae cause the majority of the damage (Figures 4.5 A, 4.6 A), so the distribution of larvae between flower and fruit stages is likely to be the best indicator for the timing of damage. At lower thrips densities (<5 adult thrips per flower), thrips larvae were concentrated in the late flowering and green fruit stages (Figure 4.8 C), so damage would occur at early stages of fruit development, 20 - 30 days before harvest, as suggested by Steiner and Goodwin (2005a). However, when thrips density was high (e.g. >11 adult thrips per flower), larvae were present in similar numbers throughout fruit development (Figure 4.8 D), so the damage could occur right up to harvest rather than mainly in the early fruit stages. The change in distribution observed as thrips density increases may result from competition for resources and interference between thrips at higher thrips densities, which would force thrips out of flowers into less favoured habitats (Crespi & Taylor, 1990). Both adult and larval thrips can move between flowers and fruit (Kirk, 1985b) and the thrips density on a particular flower or fruit could be influenced by that in surrounding flowers and fruit (with thrips hatching at different times), which would also contribute to the thrips density during the later stages of fruit development. In addition, thrips distribution on strawberry changes with pollen availability and predator establishment (Shakya *et al.*, 2010). There are proportionally more larvae on the later fruit stages when there is less pollen, as would occur later in the season when there are fewer flowers and more thrips. Further biological data on the movement of thrips larvae between strawberry parts, at different thrips, flower and *N. cucumeris* densities are required to explain the observed changes in their distribution.

The later timing of bronzing in fruit development predicted at higher thrips densities helps to explain why thrips density per flower correlated best with bronzing on white fruit when both were measured at the same time (Table 4.3). The more even distribution of larvae at higher thrips densities suggested that over 30% of the damage on white fruit (at the turning pink stage) occurred over the previous 1-2 weeks, so damage reflected current

thrips densities better than those of 3-4 weeks previously (when those white fruit would have been flowers). Another possible explanation is that thrips populations are often increasing at times when damage is occurring, which weights the timing of damage towards the later stages. For example, mean numbers of adult thrips per flower increased from <1 to about 13 over a five-week period from mid-July to mid-August 2011 in field 2 (Figure 4.11 A) and the adults alone that were present in mid-August (without subsequent larvae) could have caused damage that would not have been predicted from the low numbers of thrips in mid-July. Empirical evidence from the field shows that the assessment of thrips density in mid-aged flowers cannot reliably predict thrips damage on the white fruit (3-4 weeks later), or red fruit (about 5 weeks later) that develop from those flowers, because thrips density may increase and do further damage in between (Table 4.3), whereas there was good correlation between thrips density in flowers and damage on white fruit that develop from those flowers in experiments where other parameters were controlled (e.g. Figure 4.4). As field conditions are uncontrolled, the assessment of adult thrips density in flowers in the field is considered simply as a relative measure of thrips density that reflects relative fruit damage at that time. Published EILs and DTs have also been developed by comparing thrips density in flowers with fruit damage at the same time (see references in Table 4.1).

Quantification of realistic EILs for bronzing on strawberry fruit is difficult in commercial fields, where there is a complex relationship between bronzing and environmental conditions, cultural techniques, pest or diseases species present and control treatments applied (Polito *et al.*, 2002). Key to developing EILs is the quantification of the amount of fruit bronzing that would result in economic loss, but this is not included in most studies. Steiner and Goodwin's (2005a) damage threshold of five or more *F. occidentalis* was based on moderate to high fruit damage (>20% of the fruit surface bronzed). Coll *et al.* (2007a) incorporated economic loss into their EIL by quantifying weight loss as a result of thrips damage, which did not apply in this study because fruit were downgraded on bronzing before weight loss was affected. They also showed that different markets had different tolerance for damage, specifying 30% fruit surface bronzing as the damage threshold for the local market in Israel and a requirement for uniform size and colour for the export market (bronzing unspecified), resulting in EILs of 24 and 10 motile (adults and larvae) thrips per flower for local and export markets respectively. At the start of my study it was assumed that fruit for the UK market were downgraded if

bronzing was observed around more than a few seeds (e.g. up to five seeds), based on discussions with growers and advisors, which was reflected in the damage scale used in 2011. In reality this turned out to be more of an aspirational, rather than an actual, threshold as bronzing up to about 10% of the fruit surface was tolerated without down-grading of fruit in a commercial pack-house (Figure 4.10). Some of this discrepancy between observed and perceived damage thresholds may arise because the intensity of bronzing was not considered in this study, so the slightest bronzing was scored (observed carefully with a lens and a light) that may not have been recognised on red fruit being graded rapidly by staff in a commercial pack-house. The EILs in this study are considered conservative as the assessment of fruit bronzing was carried on red fruit in the pack-house, whereas white fruit was assessed in the field, where fruit bronzing shows up more clearly.

The EIL defined from damage assessed on pack-house fruit equated to about five adult thrips per flower in the absence of predatory mites in a controlled experiment (Figure 4.4), which was within the range of published thresholds on strawberry (Table 4.1) and the same as that identified by Steiner and Goodwin (2005a) in Australia when adult thrips were sampled. The threshold is considered conservative, not only because white fruit were assessed (see above) but also because females were used in the controlled experiment, which would have resulted in proportionally more larvae and therefore more damage, than if a mixed-sex population had been used. The sex ratio of *F. occidentalis* in strawberry flowers from the field was about 3: 1 female: male, identified from alcohol samples ($n > 2000$, Chapter 3). Despite this, the same EIL (five adult thrips per flower) was identified in two commercial crops of the same cultivar, on different farms, with relatively few predatory mites, in the same year (Figure 4.11). Further support for this EIL comes from three crops where there was no crop loss due to thrips damage throughout the season while thrips densities remained below the threshold (Table 4.4). The similarity between the EILs identified in different countries with different climates is perhaps rather surprising. A possible explanation for this is that the growing methods, planting densities and cultivars used in commercial strawberry crops are similar between countries where there is regular exchange of information and plants in a global market, and because the growing conditions between countries are more similar for protected crops.

The predatory mite *N. cucumeris* is a key component in the control of pesticide-resistant *F. occidentalis* on strawberry (Rahman *et al.*, 2012). The reduction in strawberry fruit bronzing resulting from *N. cucumeris* in this study (Figures 4.5, 4.6) is similar to that

predicted by Shakya *et al.* (2010), based on *N. cucumeris* predation rates on thrips larvae. The predatory mites prevented nearly all damage at a thrips density of four adult thrips per flower (Figure 4.5), which is further evidence that low levels of thrips (below the EIL of 5 adult thrips per flower) can be managed with predatory mites. Shakya *et al.* (2010) estimated that the EIL could be relaxed by one or two motile (adults and larvae) thrips, with and without pollen respectively, for each predatory mite present per sampling unit, which is similar to that predicted by this study, of 1.6 adult thrips per flower per predatory mite (see 4.3.2.3). The field data also indicate increased thresholds with increasing *N. cucumeris* establishment, but the relaxation of the EIL according to numbers of predators per fruit could not be tested as the predatory mites were not counted (only presence/absence data were recorded). The field data should be viewed with caution as there was insufficient replication between farms and cultivars to draw conclusions and relatively few predatory mites were identified to species, although 90% were *N. cucumeris* in the small sample of mites identified ($n = 20$). As the identification of predatory mites is time consuming and requires specialist knowledge and equipment (Zhang, 2003), it is not practical for most growers, so it would be useful to test whether it would be sufficient to sample predatory mite density without identifying them to species for the purpose of estimating an EIL. Many naturally occurring or naturalised generalist predators, such as *N. californicus*, feed on thrips in addition to their preferred prey (Walzer *et al.*, 2004), so identification may not be necessary. *Neosieulus californicus* is more frequent on leaves than on flowers and fruit (Fitzgerald *et al.*, 2008), so may be less prevalent than *N. cucumeris* in flower samples. Even where regular releases of *N. cucumeris* had been made, field distribution was patchy, so the distribution of predatory mites must be considered as well as the numbers of predators per flower, as an even distribution of *N. cucumeris* is a key factor in preventing thrips increase (Jacobson *et al.*, 2001b). Further data are required to determine the numbers or percentage cover of predators required to reduce strawberry fruit damage in the field and to determine a reliable sampling method for the predatory mites. This study has shown the importance of *N. cucumeris* in reducing thrips bronzing, so predator establishment must be taken into account within EILs. Early intervention risks disrupting the predator establishment and increasing pesticide resistance, which would result in increased numbers of thrips.

When the density of adult thrips reaches a high enough density they can cause sufficient bronzing on their own (i.e. without the subsequent damage caused by their

larvae) that will result in fruit down-grading. This damage cannot be reduced significantly by *N. cucumeris*, which only feed on larvae. This upper threshold was not tested experimentally, but there is evidence that it may be around 11-12 adult thrips per flower in UK crops: high levels of *N. cucumeris* (5 per fruit) reduced fruit bronzing below economically damaging levels at eight adult thrips per flower (Figure 6.5 A), suggesting that the upper threshold is higher than eight; six adult thrips per flower (without subsequent larvae) caused fruit bronzing around 5% of the fruit surface (around 15 seeds, Figure 4.7), suggesting that 12 adult thrips per flower would result in about 10% fruit surface bronzing (the EIL); and the highest EIL observed in a field with good predatory mite establishment was 11 adult thrips per flower (Table 4.4). Therefore an upper threshold of 11 adult thrips per flower is suggested (see Table 4.5), as few growers or advisors would risk higher thrips numbers, but this could be tested further in controlled experiments.

There were several important factors that were not incorporated into the EILs in this study. Seasonal market changes were not considered in the EIL, which was tested at times when 10-15% of the fruit was being down-graded due to fruit bronzing (S. Clarke, pers. comm, 2012) and further data are required to test how the threshold might change according to the availability of good quality fruit. When there is a glut of undamaged fruit, the EIL might be lower as only the very best quality fruit would be selected for sale. Climatic conditions may affect fruit damage. More damage was observed on chrysanthemum at longer daylength (De Jager *et al.*, 1997), which may be the result of increased feeding activity (Whittaker & Kirk, 2004). Relatively more fruit bronzing per thrips was observed on strawberry in hot, dry conditions (daytime temperatures of 35°C vs 25°C) in Australia (Steiner & Goodwin, 2005a). As a result, EILs may vary between seasons, regions, farms, or even within fields, where temperatures can be significantly higher near the top of sloping fields compared to the bottom (see Chapter 3). There were insufficient data to draw conclusions on the relative susceptibility of different cultivars to thrips damage, but the limited data available showed similar susceptibility to damage between the two cultivars tested (cvs. Camarillo and Finesse).

Orius spp. were not tested in this study as few growers in W. Midlands were releasing it and only a few naturally occurring *Orius* sp. were observed in fields at the end of the season (<1% of flowers with *Orius*). *Orius* spp. achieve spectacular control of both adult and larval *F. occidentalis* when well established (Sampson *et al.*, 2011) and where naturally occurring *Orius* spp. are widespread, the EIL is rarely reached unless harmful

pesticide treatments are made (Coll *et al.*, 2007a). *Orius* spp. are estimated to relax EILs by 40% (Shakya *et al.*, 2010), so *Orius* spp. should be incorporated into the EIL if growers start to use them effectively. Other naturally occurring predators were observed in the field, such as the common flower bug (*Anthocoris nemorum*), and these contribute to control. Shakya *et al.* (2010) incorporated the presence or absence of pollen into their decision-making tool, as predators feed on more thrips in the absence of pollen. The presence of pollen was not included in this study as it is available nearly continuously in everbearer strawberry cultivars. Pollen is a key factor affecting the establishment and population growth of predatory mites and *Orius* spp. in strawberry, so although the predation rate per predator may decrease in the presence of pollen, the resulting increase in population growth is likely to result in an overall increase in predator numbers and total predation.

The recommended sample sizes required to estimate thrips populations using visual assessment vary in the literature between 10-50 flowers per plot in strawberry (García-Mari *et al.*, 1994; Laudonia *et al.*, 2000; Linder *et al.*, 2000; Steiner & Goodwin, 2005a). This study supports the sampling of 10 flowers per area of interest based on the aggregation index ($b = 1.12$), which is the lowest estimate, but similar to published indices for adult thrips in strawberry of 1.26 to 1.42 (García-Mari *et al.*, 1994; Linder *et al.*, 2000; Steiner & Goodwin, 2005a). The relatively low aggregation index could be the result of the large sample size, combining samples from different dates, which would have included a significant proportion of other thrips species early in the season (see Chapter 3), which can have lower aggregation indices (Steiner & Goodwin, 2005a). The sample size required increases when larvae are included as they are more aggregated and less mobile than adults (Steiner, 1990). The choice of sample size will vary according to time available, thrips density and a grower's attitude to risk. The sampling of 10 flowers from an area of interest would usually be sufficient to estimate adult thrips density in most situations, but different areas of a crop should be sampled as thrips numbers vary considerably within fields (see Chapter 3). Sampling plans should be tailored to specific fields based on local knowledge as pest hot-spots (areas with higher pest density) are usually known by growers. As thrips density and fruit bronzing change rapidly through the season, weekly sampling is essential to make appropriate management decisions so that fruit damage can be prevented. Although not tested experimentally, an increase in thrips density per flower and fruit damage was often observed at the end of flower flushes, when

the thrips concentrated into fewer flowers and moved onto fruit (Figure 4.11). Future damage cannot therefore be predicted from thrips density alone but has to take account of changes in flower density. Further data are required to quantify this experimentally, but extra vigilance is advised when flower density is declining. The thresholds developed in this study depended on careful sampling of adult thrips in flowers of a specific age and position. Inaccurate sampling would underestimate thrips numbers and make the thresholds invalid. Training of staff (using methods described in Chapter 2) and checking of thrips counts by eye against alcohol samples would be a worthwhile investment for growers at the start of each season.

Flower sampling gave a better correlation with thrips damage on fruit than trap catches. Traps can be less reliable for estimating a population than counts of thrips in flowers as they reflect activity as well as population density, so are more influenced by environmental conditions such as wind speed and temperature (Shipp & Zariffa, 1991; Steiner & Goodwin, 2005b). Flower sampling gives a more direct relationship to fruit damage as it naturally accounts for any changes in flower density that affect numbers of thrips per flower even when there is no change in the thrips population. Also because flower counts give an immediate measure whereas trap catches are delayed. Binomial sampling was assessed as it could provide growers with a faster sampling method (Steiner & Goodwin, 2005a). In this study, the numbers of thrips per flower and risk of damage, increased rapidly once 100% flower occupation had been reached, which equated to about 4 adult thrips per flower, which is just below the EIL without predatory mites of 5 adult thrips per flower. With good establishment of natural enemies and thus higher thresholds, binomial sampling will not be of use around the threshold. One possible option is to use binomial sampling until 100% flower occupancy as an alert that EILs are approaching, and then change to counts of adult thrips per flower to give a more detailed estimate, but this would have to be tested further in commercial crops.

The EILs identified for *F. occidentalis*, with and without predatory mites, in controlled studies and in commercial strawberry are remarkably consistent in this study (Table 4.4), but they are mainly derived from one cultivar (cv. Camarillo) and from one region of the UK. Further data are required to test how factors such as the market, cultivar and climate affect EILs in different regions of the UK. The thresholds and actions suggested in Table 4.5 can be used only as a rough guide, to be amended according to experience in different markets, seasons, cultivars, farms or fields. However, these conservative thresholds (based

on damage on white fruit), suggest that higher numbers of thrips may be tolerated without damage than is currently considered acceptable to growers. Many growers routinely spray chemical insecticides when thrips density is below one adult thrips per flower. The adoption of thresholds for timing treatments is likely to result in reduced frequency of potentially harmful pesticide applications that can reduce predator numbers and so cause a resurgence of *F. occidentalis*. Reduced spraying also delays the development of pesticide resistance.

Table 4.1. Published action thresholds (ATs), damage thresholds (DTs) and economic injury levels (EILs) for strawberry, based on numbers of thrips per flower where, m = mixed active thrips stages and A = adult thrips.

| Reference | Threshold: no. of thrips per flower | Type of threshold | Thrips stage(s) | Thrips species | Comment |
|-------------------------------|-------------------------------------|-------------------|-----------------|--------------------|-------------------------|
| Linder <i>et al.</i> , 2000 | 3-6 | DT | m | mixed ^a | Switzerland |
| Steiner & Goodwin, 2005a | 5 ^b | DT | A | WFT | Australia |
| | 10 | DT | m | WFT | |
| Grasselly, 1995 | 8-10 | DT | m | WFT | France |
| Shakya <i>et al.</i> , 2010 | 10 | EIL | m | WFT | Israel, no <i>Orius</i> |
| | 14 | EIL | m | WFT | With <i>Orius</i> |
| Coll <i>et al.</i> , 2007b | 10 | EIL | m | WFT | Export market |
| | 24 | EIL | m | WFT | Local market |
| Laudonia <i>et al.</i> , 2000 | 7-15 | AT | m | WFT | Italy |
| | 15-20 | EIL | m | WFT | Italy |

^a Species did not include *F. occidentalis*

^b AT = 5 adult thrips per flower in 40% of flowers

Table 4.2. Progression of semi-protected strawberry (cv. Camarillo) from white bud (petals visible) to red fruit from 29 June 2011, at an average 15.7°C (n = 6 flowers or fruit).

| Flower stage | Description of stage | Time taken for each stage (days) |
|------------------|--|----------------------------------|
| White bud | Petals showing but not open. | 4.0 ± 0.4 |
| Open flower | All petals open and present. | 4.8 ± 0.7 |
| Senescent flower | Petals dropping, one to five petals present. | 2.5 ± 0.7 |
| Button fruit | No petals, receptacle elongating, seeds visible. | 2.0 ± 0.3 |
| Green fruit | Green seeds covering a larger area than flesh. | 13.5 ± 0.5 |
| White fruit | White flesh covering a larger area than seeds. | 7.7 ± 0.7 |
| Pink fruit | Fully swollen white fruit with some red areas. | 8.0 ± 0.7 |
| Red fruit | Ripe red fruit that was harvested after one day. | 1.0 ± 0.0 |

Table 4.3. Polynomial (linear and quadratic) regression of fruit damage (seeds surrounded by bronzing) on thrips density (adult thrips per flower) on the same date, then of fruit damage on thrips density in previous successive weeks, in two semi-protected strawberry crops (cv. Camarillo) (fields 2 and 3, Table 2.1). Data were collected weekly from May to September 2011. There were two plots in each field, with ten flowers and fruit sampled weekly per plot. Analysis was of log-transformed data. The regression had linear and quadratic components, with fruit damage increasing rapidly at lower thrips numbers but levelling off at high thrips numbers.

| Time lag between flower samples and fruit damage assessments | Field 2 | | | Field 3 | | |
|---|--------------|--------|-------|--------------|--------|-------|
| | $F_{(d.f)}$ | P | R^2 | $F_{(d.f)}$ | P | R^2 |
| On the same dates | 57.6 (2, 11) | <0.001 | 89.7 | 68.3 (2, 33) | <0.001 | 79.4 |
| Damage after 1 week | 35.6 (2, 10) | <0.001 | 85.2 | 54.5 (2, 33) | <0.001 | 75.3 |
| Damage after 2 weeks | 13.0 (2, 9) | 0.002 | 68.6 | 42.4 (2, 33) | <0.001 | 70.3 |
| Damage after 3 weeks | 6.2 (2, 8) | 0.02 | 51.2 | 28.3 (2, 33) | <0.001 | 60.9 |
| Damage after 4 weeks | 2.0 (2, 7) | 0.21 | 17.8 | 12.2 (2, 33) | <0.001 | 38.3 |
| Damage after 5 weeks | 0.7 (2, 6) | 0.55 | 0 | 6.7 (2, 33) | 0.003 | 23.7 |
| Damage after 6 weeks | 1.5 (2, 5) | 0.31 | 12.0 | 2.2 (2, 33) | 0.13 | 6.1 |

Table 4.4. Economic injury levels (EILs) observed in commercial semi-protected strawberry crops, derived from regression analysis of fruit damage (seeds surrounded by bronzing) on thrips density (adult thrips per flower) to predict the thrips density causing damage over 10% of the fruit surface. Samples were taken on the same dates, which previously gave the best correlations (Table 4.3). The mean number of adult thrips per flower when the thrips populations were at their peak and the percentage of fruit supporting predatory mites (*Neoseiulus* spp.) are shown. N/A = Not available.

| Location (year) | Cultivar | Peak sampling density ^a | % fruit with predators | R^2 | P | EIL | Data source: field, (ref. ^b) |
|--|-----------|--|------------------------------|-------|--------|----------------|---|
| Stafford (2011) ^c | Camarillo | 12.9 | 4% | 90% | <0.001 | 5.0 ± 0.3 | Field 2 (4.2.5.1) |
| Tamworth (2011) ^c | Camarillo | 10.3 | 42% | 79% | <0.001 | 5.0 ± 0.3 | Field 3 (4.2.5.1) |
| Stafford (Sept 2012) | Camarillo | 6.0 | 5% | 88% | <0.001 | 6.3 ± 0.4 | Field 10 (6.2.6.2) |
| Tamworth (May 2012) | Finesse | 12.5 | 62% | 65% | 0.009 | 8.7 ± 1.3 | Field 7 (3.2.5) |
| Tamworth (Aug 2012) | Camarillo | 18.5 | 60% | 63% | <0.001 | 8.8 ± 0.4 | Field 3 (3.2.5) |
| Tamworth (Aug 2012) | Camarillo | 17.1 | 72% | 62% | <0.001 | 10.6 ± 1.1 | Field 4 (6.2.7) |
| The following crops did not have sufficient thrips to cause fruit downgrading: | | | | | | | |
| Stafford (2012) | Camarillo | 1.0 | 72% | - | - | N/A | Field 9 (3.2.5) |
| Tamworth (2012) | Finesse | 3.1 | N/A | - | - | N/A | Field 5 (3.2.5) |
| Tamworth (2013) | Finesse | 1.0 | N/A | - | - | N/A | Field 3 (d) |

^a The highest mean number of thrips per flower recorded over the whole season.

^b Reference to the experiment from which the data came.

^c 2011 thresholds were derived from weekly samples taken throughout the season.

^d Data were used from samples of 21 plots (n = 40 flowers and 20 fruit per plot) spread over the field from an experiment that was not reported in this study.

Table 4.5. A decision table illustrating the adjustment of economic injury levels (EILs) and possible thrips control actions according to thrips density (numbers of adult thrips per flower) and predatory mite establishment (numbers of *N. cucumeris* per fruit), within an Integrated Pest Management (IPM) programme in UK semi-protected strawberry in the West Midlands, where: X = numbers of *N. cucumeris* adults per fruit. EIL = economic injury level (adult thrips per flower).

Instructions – count the number of adult thrips per flower in 10 medium aged flowers selected from the top of the plant, and the numbers of adult predatory mites per fruit per 10 white fruit, then select the appropriate action according to the relevant boxes for thrips and predator density in the table below.

| | | Predatory mite level | |
|--------------|---------------------------------|---|--|
| | | Predatory mites present on the majority of fruit. Adjust the EIL according to: $EIL = 5 + 1.6 X$ | Poor distribution (<50% of fruit with mites) of predatory mites on fruit: $EIL = 5$ |
| Thrips level | <5 adult thrips per flower | ➤ Monitor next week. | ➤ Release <i>N. cucumeris</i> . ➤ Monitor next week. |
| | 5 to 11 adult thrips per flower | ➤ Apply compatible spray treatment if EIL (above) is reached. ➤ Monitor next week. | ➤ Apply compatible spray treatment. ➤ Release <i>N. cucumeris</i> . ➤ Monitor next week. |
| | >11 adult thrips per flower | ➤ Apply compatible spray treatment. ➤ Check predator numbers. ➤ Monitor next week. | ➤ Apply compatible spray treatment. ➤ Release <i>N. cucumeris</i> . ➤ Monitor next week. |

Note: IPM assumes that no incompatible spray-treatments with a long residual action have been used. If *Orius* spp. are present, the EILs could be increased further. Earlier treatment may be required if the treatment is slow acting.

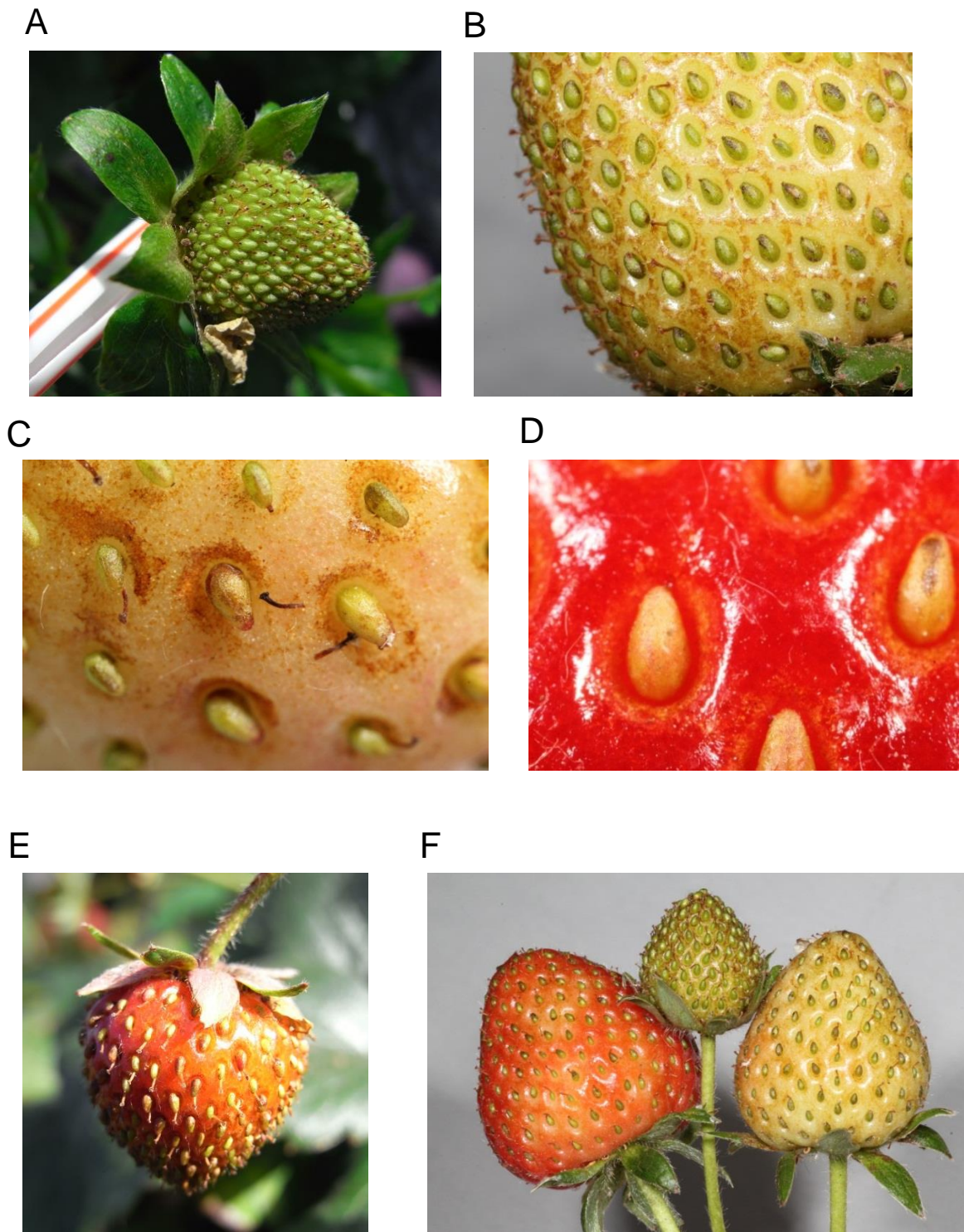


Figure 4.1. Photographs of types of damage seen on strawberry fruit, showing: (A) bronzing on the flesh between seeds at the earliest stages of green fruit development, (B) a netting pattern on white fruit resulting from earlier damage, (C) later bronzing caused by thrips feeding in the wells surrounding seeds in white fruit, (D) and bronzing surrounding the seeds on red fruit, (E) 'seedy' appearance of a severely bronzed red fruit and (F) typical thrips damage on green, white and red fruit.

A



B



Figure 4.2. Photographs of strawberry (cv. Finesse) from a semi-protected field that was heavily infested with thrips showing (A) a damaged flower truss where flowers are discoloured and damage is visible on the young green fruit and (B) discarded fruit due to thrips bronzing.

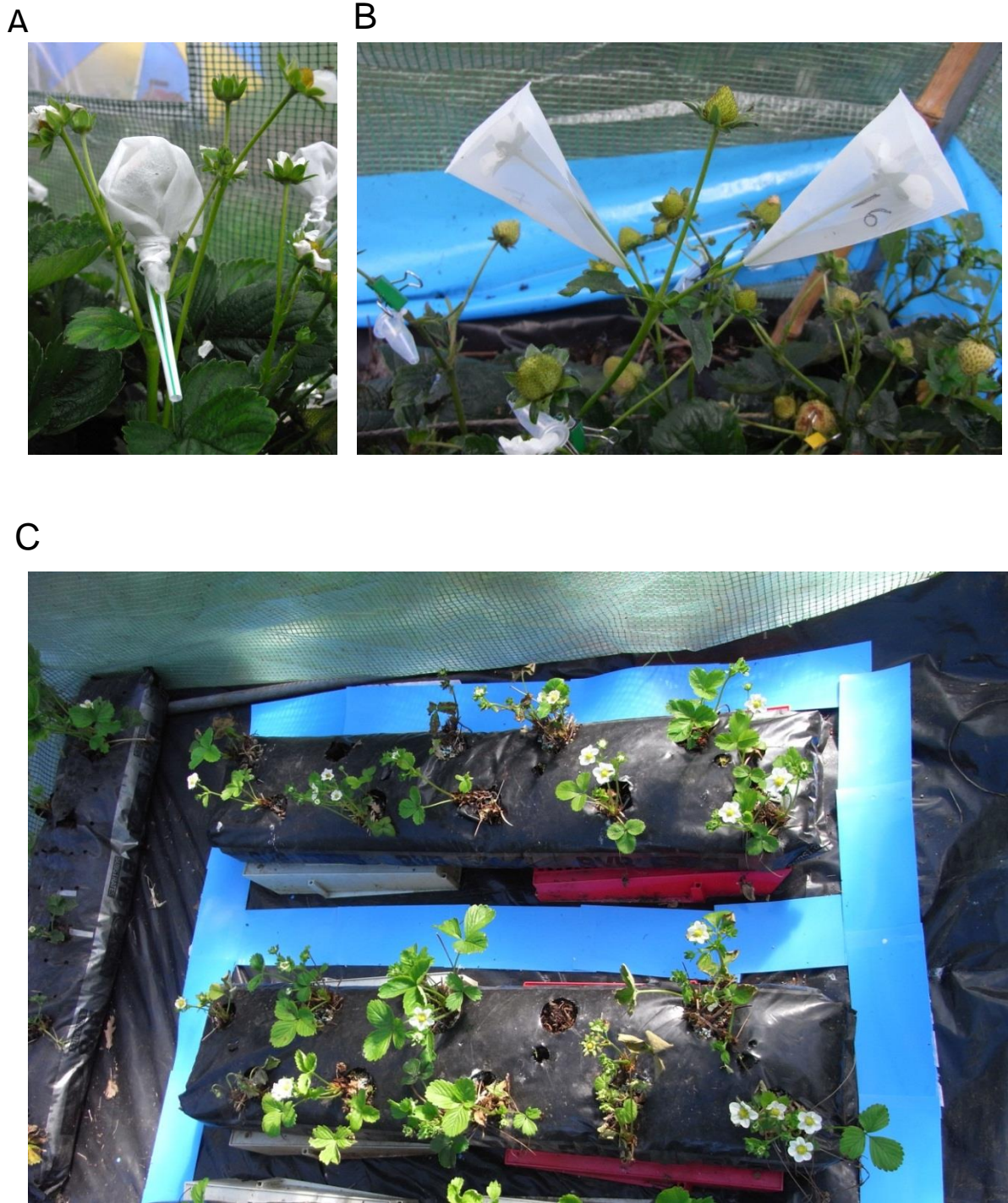


Figure 4.3. Photographs showing experimental methods: (A) caged flowers for the damage experiment in 2011, (B) caged flowers for the damage experiments in 2013 and (C) the lay-out of a single plot, with and without predatory mites (taken before the experiment had started in the spring).

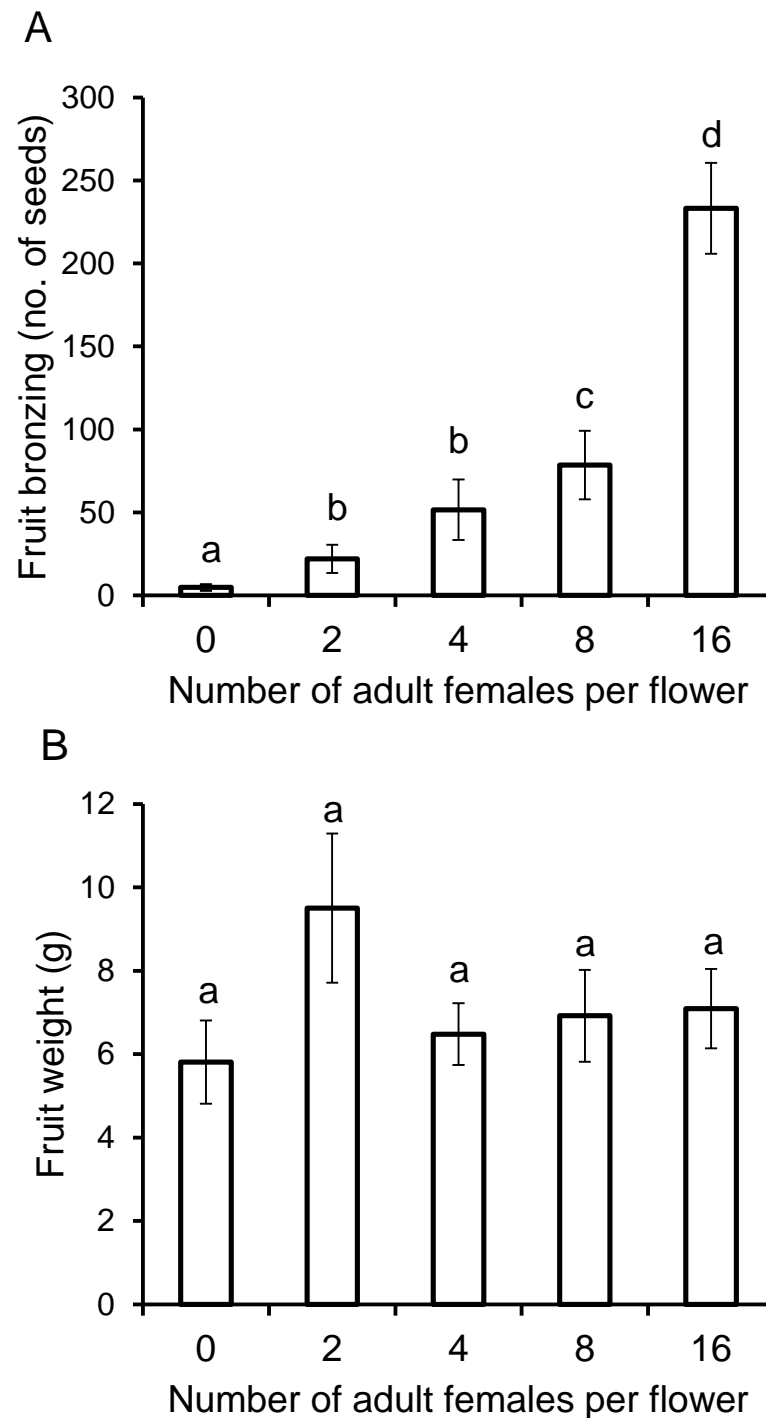


Figure 4.4. (A) The mean numbers \pm SEM of seeds surrounded by bronzing per fruit ($F_{(4, 79)} = 36.86$, $P < 0.001$) and (B) the mean fruit weight (g) \pm SEM ($F_{(4, 62)} = 1.7$, $P = 0.15$), following infestation of strawberry flowers with different numbers of adult female *F. occidentalis* in a semi-protected strawberry crop (cv. Camarillo) ($n = 17$). Means with the same letter are not significantly different (Tukey's test, $P > 0.05$). Analysis was on log-transformed data whilst the chart shows untransformed data.

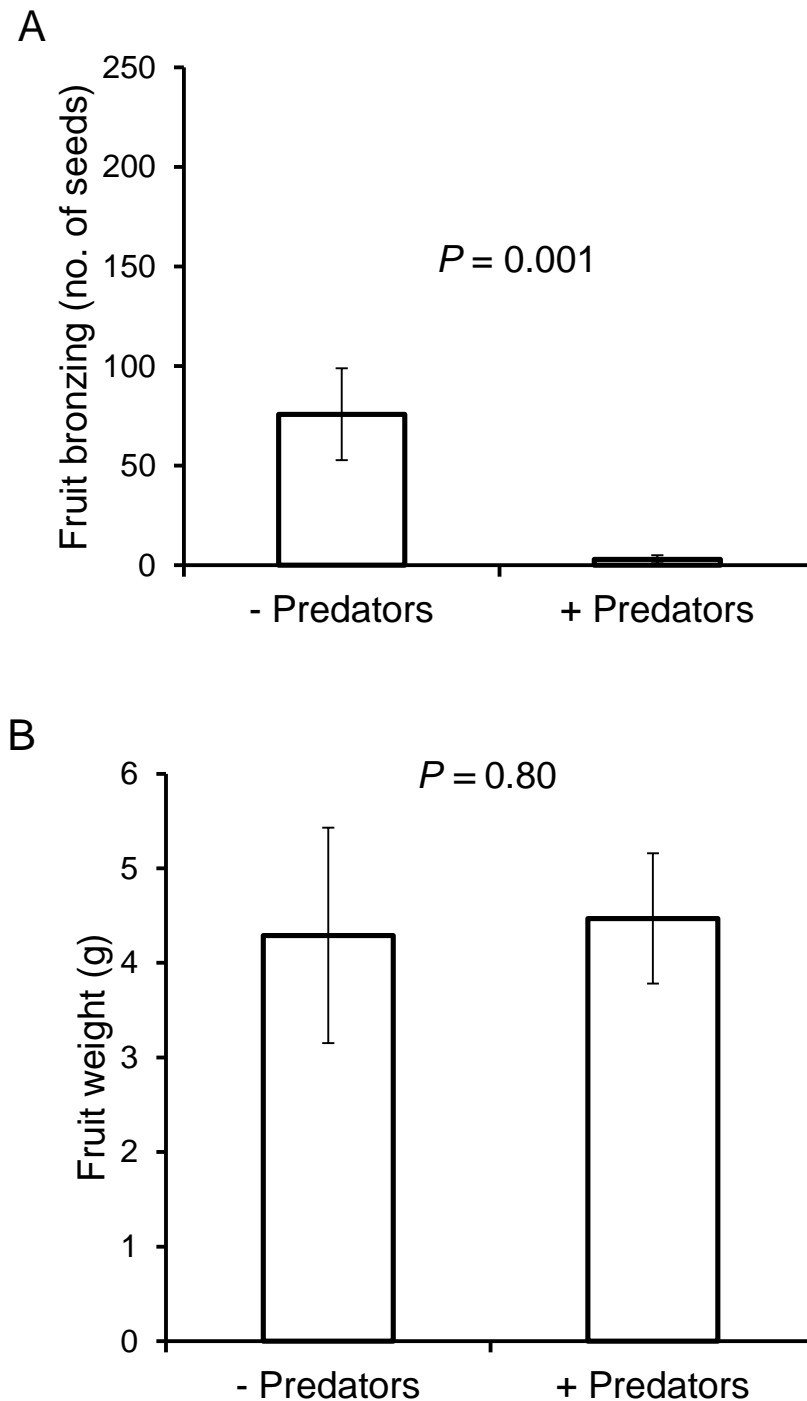


Figure 4.5. (A) The mean numbers of seeds surrounded by bronzing per fruit \pm SEM ($F_{(1, 6)} = 37.7$, $P = 0.001$) and (B) the mean fruit weight (g) \pm SEM ($F_{(1, 6)} = 0.08$, $P = 0.80$), following infestation of strawberry flowers with four adult female *F. occidentalis* with or without the predator *N. cucumeris* in a semi-protected strawberry crop (cv. Camarillo) ($n = 4$ blocks). Analysis was on log-transformed data whilst the chart shows untransformed data.

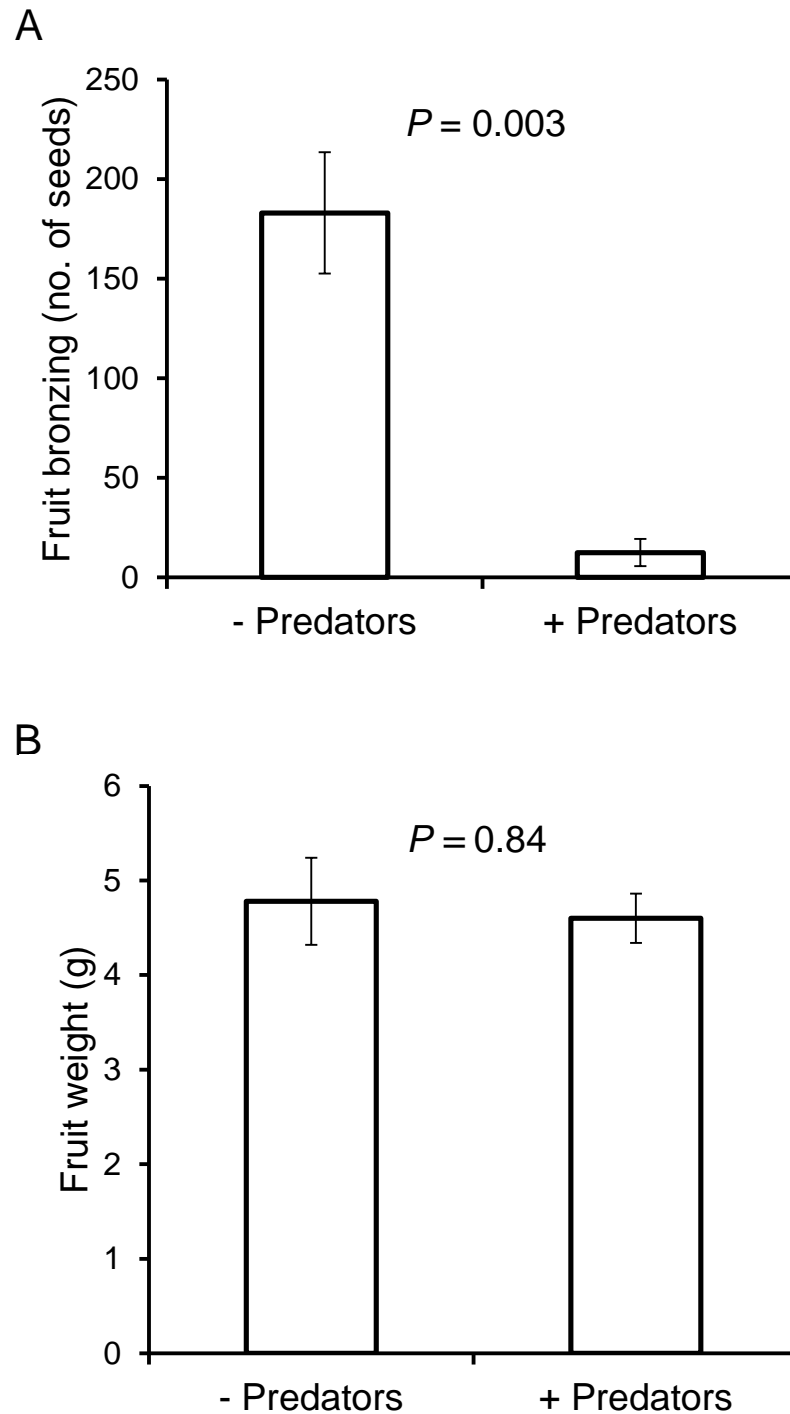


Figure 4.6. (A) The mean numbers of seeds surrounded by bronzing per fruit \pm SEM ($F_{(1,6)} = 75.5$, $P = 0.003$) and (B) the mean fruit weight (g) \pm SEM ($F_{(1,6)} = 0.05$, $P = 0.84$), following infestation of strawberry flowers with eight adult female *F. occidentalis* with or without the predator *N. cucumeris* in a semi-protected strawberry crop (cv. Camarillo) ($n = 4$ blocks). Analysis was on log-transformed data whilst the chart shows untransformed data.

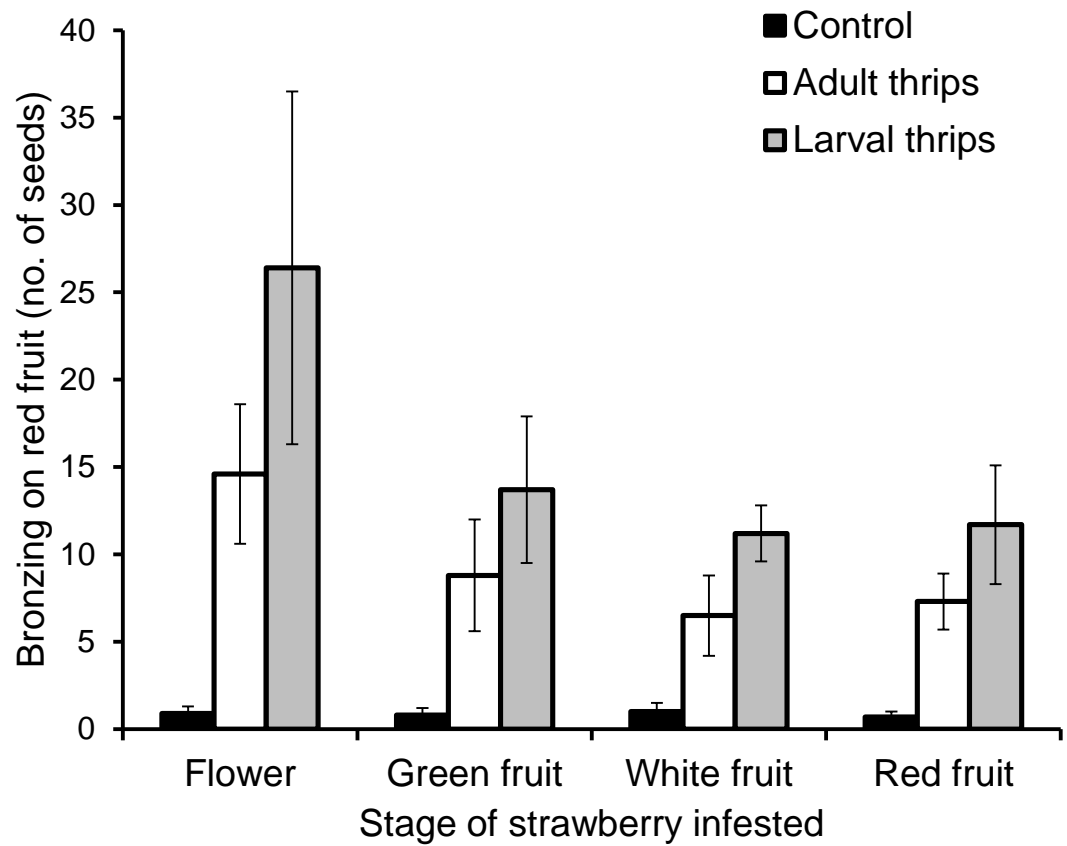


Figure 4.7. Mean number of seeds surrounded by bronzing per fruit \pm SEM, following infestation of strawberry flowers, green fruit, white fruit or red fruit for 7 days, with six adult female, or six second instar larval *F. occidentalis*, or an untreated control (cv. Camarillo) ($n = 10$ flowers or fruit). Fruit bronzing represents the numbers of seeds surrounded by bronzing on harvested red fruit. There was a significant difference between the amount of damage caused by thrips larvae, thrips adults and control treatments (two-factor ANOVA, $F_{(2,100)} = 77.6$, $P < 0.001$). There was no significant difference in susceptibility to damage between the plant stages (two-factor ANOVA, $F_{(3,100)} = 1.5$, $P = 0.23$). Analysis was on log-transformed data whilst the chart shows untransformed data.

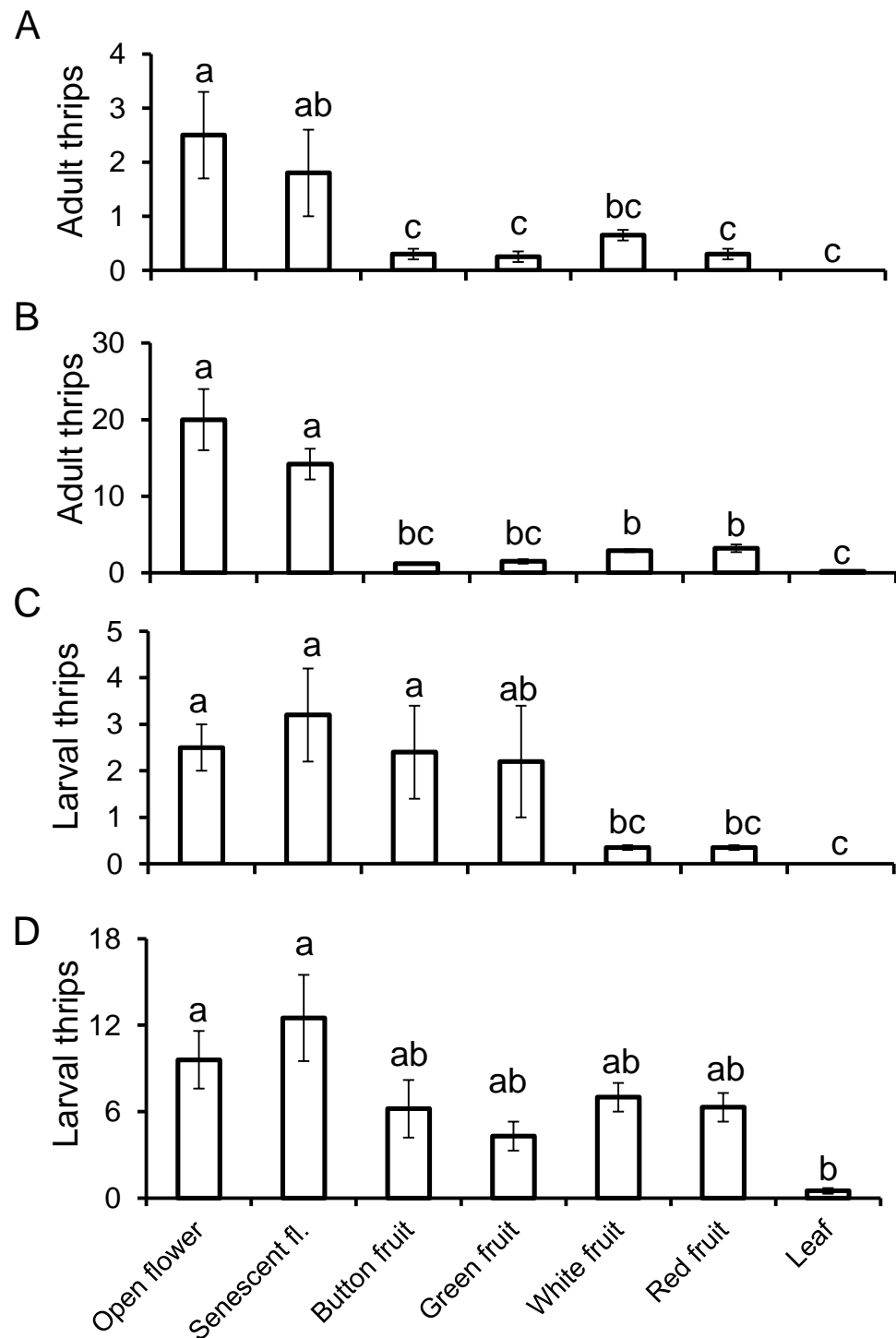


Figure 4.8. The distribution of adult thrips between plant parts in fields with (A) low thrips and (B) high thrips and the distribution of larval thrips in fields with (C) low thrips and (D) high thrips in semi-protected strawberry. Each bar is the mean number of thrips \pm SEM per plant part from two fields ($n = 10$ plant parts per field). Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42

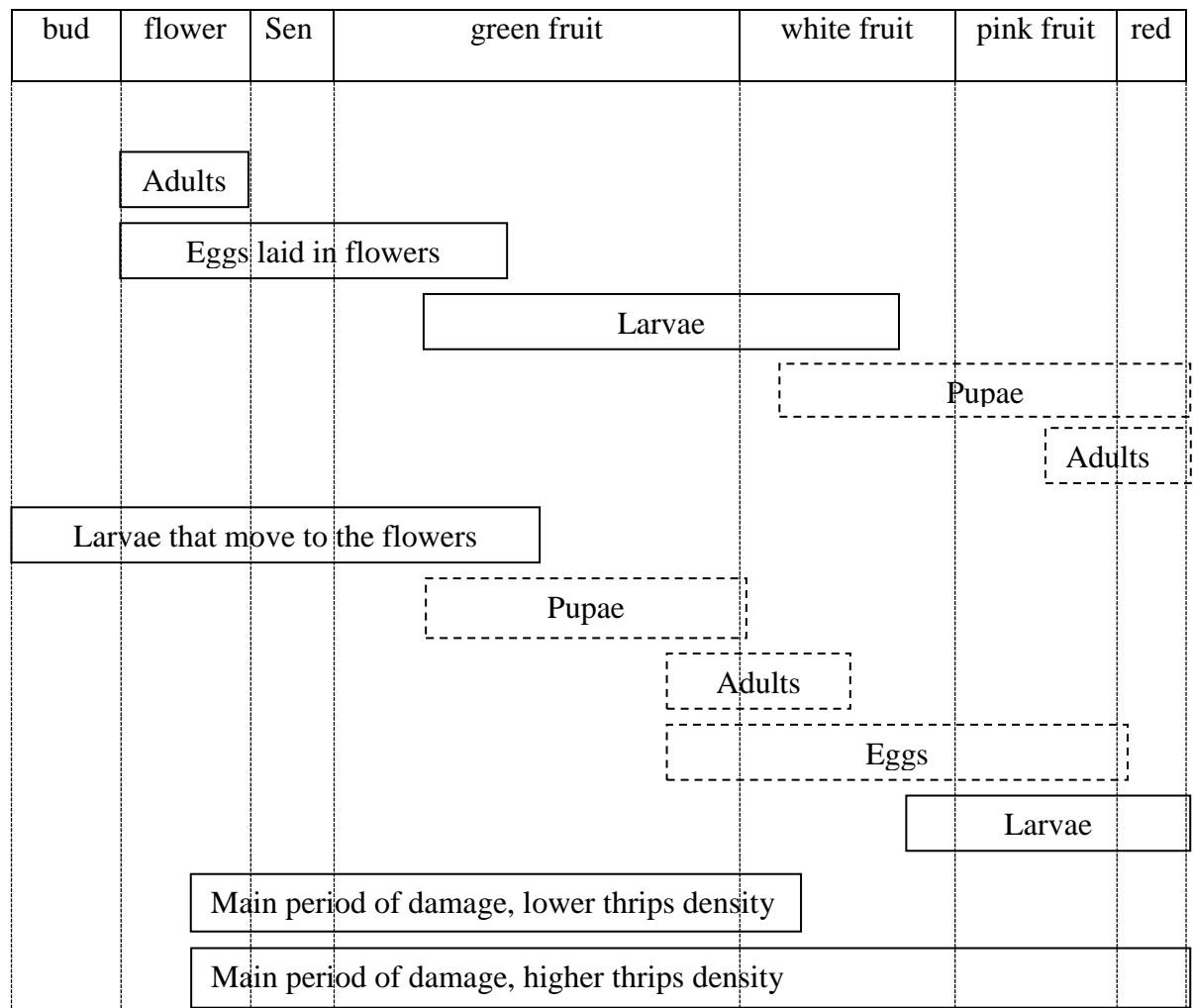


Figure 4.9. The relative timing of thrips populations and fruit damage in relation to fruit development in semi-protected strawberry (cv. Camarillo) showing the progression of flowers from bud-burst to red fruit in days, the progression of thrips from adults that lay eggs in strawberry flowers and the progression of thrips from larvae that move to the flowers from bud-burst. The predicted timing of damage is shown, based on the field distribution of thrips at low and high thrips densities. Development times for different *F. occidentalis* stages on strawberry used published data at 16°C (Nondillo *et al.*, 2008). Development stages surrounded by dotted lines may no longer be on the fruit, sen = senescent flower, red = red fruit.

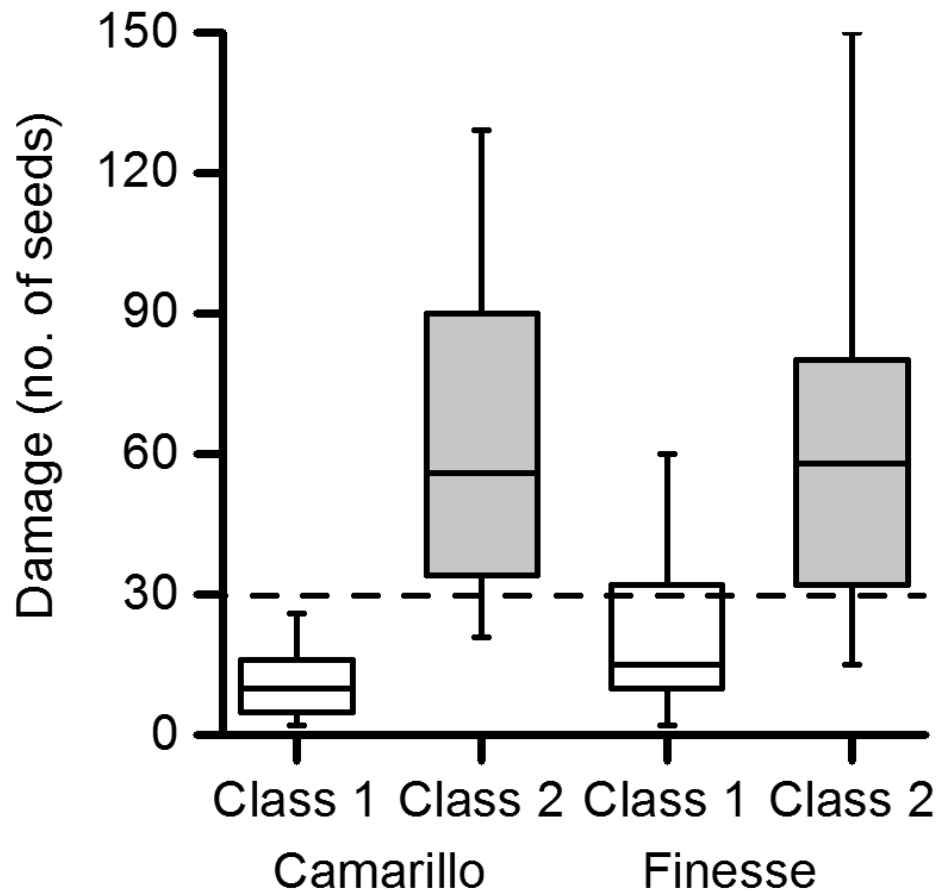


Figure 4.10. Boxplots of the amount of damage on class 1 (empty bars) and class 2 (shaded bars) strawberry red fruit for cultivars Camarillo and Finesse from the pack-house in 2012, suggesting a damage threshold of around 30 seeds (dashed line). Damage was recorded as the number of seeds surrounded by bronzing at harvest. Wide bars indicate the inter-quartile range (50% of values) and the horizontal dashed line indicates the median. The vertical lines indicate the data range excluding a few outliers of very damaged fruit in the class 2 category.

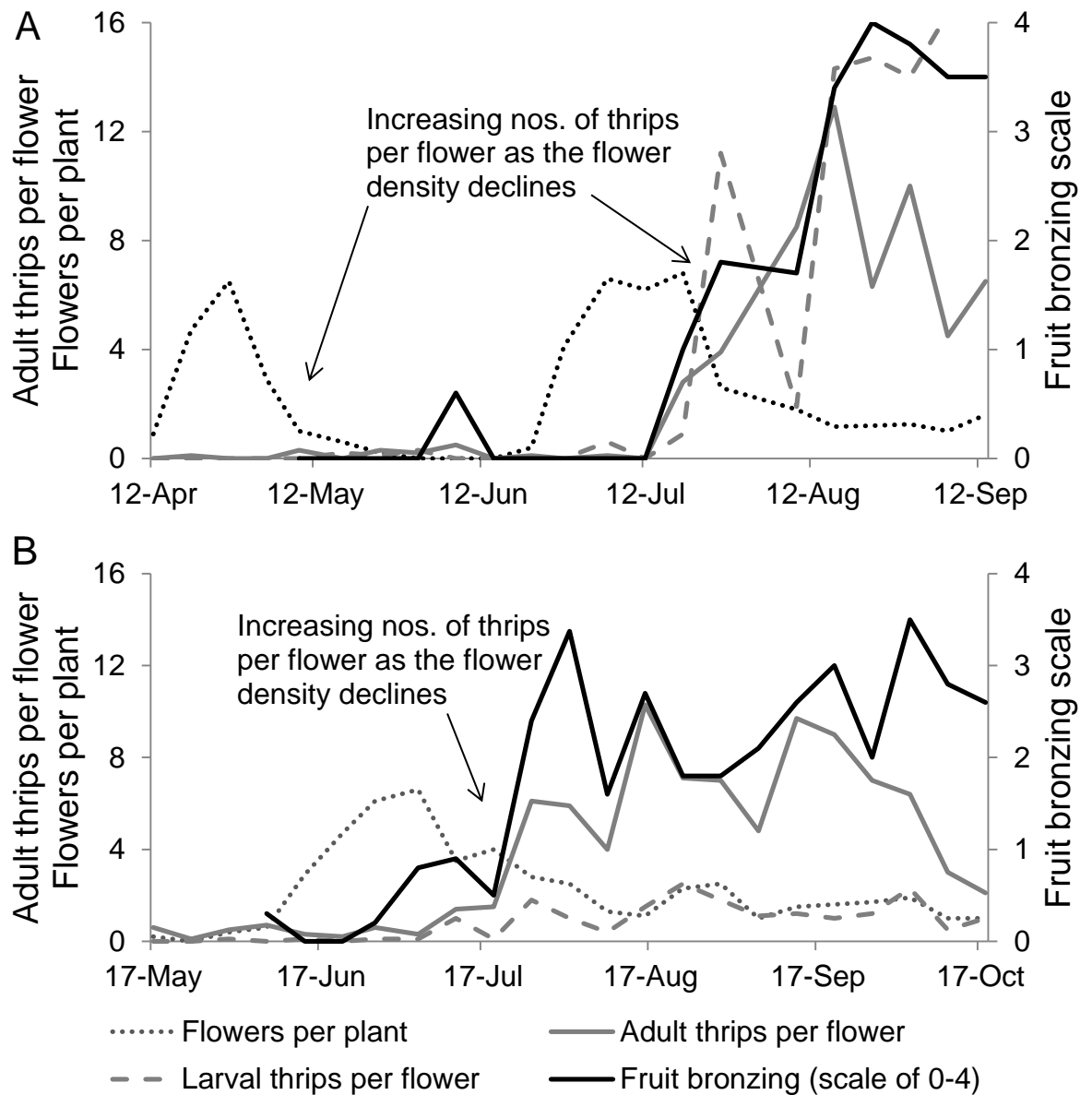


Figure 4.11. Samples taken weekly through the season (from first flowering to end of harvest), in semi-protected strawberry crops on two farms in 2011 (cv. Camarillo): (A) field 2 (Table 2.1) (B) field 3 (Table 2.1). Mean number of flowers per plant, mean number of thrips per flower and mean white fruit bronzing are shown ($n = 20$ flowers or fruit), Fruit bronzing was scored on a scale: 0 = No damage, 1 = tracking under the calyx, 2 = bronzing around 1-3 seeds, 3 = bronzing from 4 seeds to 50% of the fruit surface area, 4 = >50% of the fruit surface bronzed. Proportionally more thrips larvae were present in field 2 where 4% of fruit were infested with predatory mites than in field 3, where 42% of fruit were infested with predatory mites.

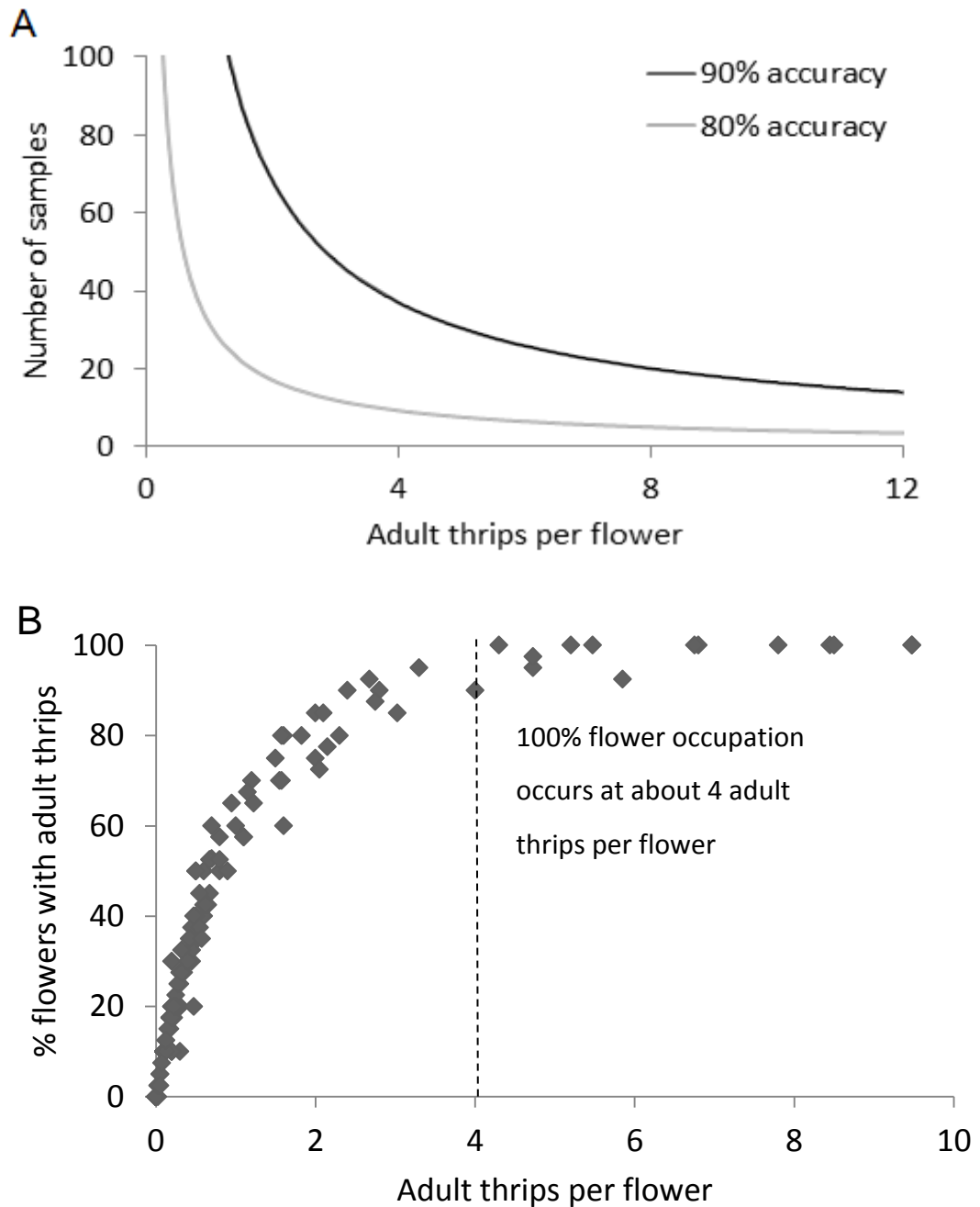


Figure 4.12. (A) Number of flower samples required for estimating thrips density in strawberry flowers (cv. Camarillo), based on the number of adult thrips per flower, to achieve 80% and 90% accuracy. (B) The relationship between numbers of adult thrips per flower and percentage of flowers infested by thrips in semi-protected strawberry (cv. Camarillo). Each data point is a mean from the field plots sampled ($n = 40$). When the mean number of adult thrips per flower (m) was adjusted to a linear relationship ($m' = m^{1-0.5b}$), the percentage of flowers infested = $-8.07 + 59.6 \times m'$.

Chapter 5

Optimising pheromone use for trapping

5.1. Introduction

Sex and aggregation pheromones are used for the monitoring, mass trapping and control of insects by eliciting various behaviours, such as activity, upwind flight, landing, orientation, arrestment and mating (Jones, 1998). Sex pheromones elicit a response only in the opposite sex whilst aggregation pheromones elicit a response in both sexes. Male *F. occidentalis* produce an aggregation pheromone, neryl (*S*)-2-methylbutanoate (Figure 5.1 A), which attracts and increases activity levels of both males and females (Hamilton *et al.*, 2005; Olaniran, 2013). It is sold commercially (Thripline_{ams}, Syngenta Bioline, Clacton, UK) for pest monitoring, and enhances trap catch, but by relatively small amounts ($\times 1.3 - \times 3$) (Hamilton *et al.*, 2005; Gómez *et al.*, 2006; Broughton, 2009). The aim of the experiments in this chapter was to try to identify factors that could improve the use of the *F. occidentalis* aggregation pheromone for trapping in the field.

The response to different dose rates of sex and aggregation pheromones varies between insect species and can affect trap catch. Many insect species use sex pheromones to locate an individual mate and land in response to specific concentrations of pheromone, with higher and lower thresholds, so it is not possible to increase trap catch by increasing the dose rate. For example, the emerald ash borer (*Agrilus planipennis*) are caught on pheromone traps at a release rate of 2.5 μg per day of (3Z)-lactone, but not at higher release rates (Ryall *et al.*, 2012). In closely related species that share the same pheromones, the dose rate can be used to identify a mate of the same species. Many species of North American tortricids share the same five pheromones (tetradecenyl acetates and dodecenyl acetates) with species differentiation dependent on the relative proportions of each (Cardé & Baker, 1984). In other species, response increases with increasing dose rate, as in the potato tuber moth (*Phthorimaea operculella*) (Larrrain *et al.*, 2007). Continuous production

of pheromone requires energy and comes at a cost, so some species vary the production rate. Pheromone production may only occur in the presence of a host plant, signalling food availability to a mate. Pheromone production may stop once a mate has been found, as in the greater grain borer (*Prostephanus truncates*) (Smith *et al.*, 1996). In the cabbage looper (*Trichoplusia ni*) both production of pheromone and responsiveness to pheromone varies through the day, peaking 6-8 hours after dark (Sower *et al.*, 1970). In the pine engraver beetle (*Ips pini*) pheromone production declines with age, so dose is an indication of fitness and may be a factor in mate selection (Miller *et al.*, 1989). Many insect species from different orders form aggregations containing variable numbers of males resulting in variable pheromone doses in the environment (Howse, 1998). Some species of Nitidulidae beetle, such as *Carpophilus antiquus*, have a negative feedback mechanism, modifying the pheromone release rate according to the amount in the environment so that the total concentration remains similar, i.e. if more beetles are present each produces less pheromone (Bartelt *et al.*, 1993b).

Limited information is available on the natural release rates of the *F. occidentalis* aggregation pheromone and it is not known whether the dose rate is critical to the response. A range of release rates have been calculated by entraining adult male *F. occidentalis* headspace odour using solid-phase micro-extraction with gas chromatography-mass spectrometry (SPME/GC-MS). These vary from 100 pg male⁻¹ h⁻¹ from groups of 5 males (Dublon *et al.*, 2008), 120 pg male⁻¹ h⁻¹ from groups of 30-60 males (Kirk & Hamilton, 2004), 300 pg male⁻¹ h⁻¹ from groups of 15 males (Dublon *et al.*, 2008) to 1 ng male⁻¹ h⁻¹ from groups of 30 males (Zhu *et al.*, 2012). Whole body washes of males failed to find the pheromone, indicating that the pheromones are produced on demand and not stored (Kirk & Hamilton, 2004). The presence of females did not affect pheromone production (Dublon *et al.*, 2008). The evidence suggests that the pheromone is produced on demand by variable amounts, which do not appear to be density dependent. As male thrips aggregate in mating swarms of varying size (Terry & Gardner, 1990), the pheromone is likely to be present in variable amounts in the environment. De Kogel *et al* (2003) found increased response of females to higher densities of males in an olfactometer experiment, although this could relate to other cues. Olaniran (2013) found more females near to filter paper discs with 5 ng than to lower and higher doses of synthetic neryl (*S*)-2-methylbutanoate, but it is not known whether trap catch of *F. occidentalis* is affected by the aggregation pheromone release rate in the field.

Insects respond to pheromones with specific chemical structures, and slight modifications to the structure or stereochemistry can change the attraction of a pheromone (Klun *et al.*, 1973; Mori, 2007). Commonly only one chiral form of the pheromone is attractive while the enantiomer is not, although sometimes the enantiomer inactivates the pheromone. In the Japanese beetle (*Popillia japonica*) (*R*)-japonilure is emitted by females to attract males, but the enantiomer (*S*)-japonilure is emitted by the related Osaka beetle (*Anomala osakana*) and stops the response to (*R*)-japonilure in male Japanese beetle, so it is involved in species recognition (Tumlinson *et al.*, 1977; Ishida & Leal, 2008). Sometimes both stereoisomers are biologically active, and are required in specific ratios, as in the ambrosia beetle (*Gnathotricus salcatus*) (Borden *et al.*, 1975), while in *Ips* species different enantiomers of ipsdienol are used by different species in the same genus (Birgersson *et al.*, 2012), and in the red flour beetle (*Tribolium castaneum*) two enantiomers are active pheromones and two are synergists (Lu *et al.*, 2011). In *F. occidentalis*, there is limited knowledge on whether the enantiomer of its aggregation pheromone, neryl (*R*)-2-methylbutanoate (Figure 5.1 B), is biologically active (Hamilton *et al.*, 2005). A racemic mix of 1:1 neryl (*S*)-2-methylbutanoate: neryl (*R*)-2-methylbutanoate reduced the trap catch compared to an untreated control in a pepper crop (Dublon, 2009), but further data are required to test whether chirality is critical to the response. Commercial sources of chemicals often contain a racemic mixture as they are easier and cheaper to synthesise. If chirality affects trap catch then the purity of the pheromone source needs to be considered.

Pheromones often comprise a unique blend of chemicals and a second volatile chemical, (*R*)-lavandulyl acetate, has been identified in the head space of male *F. occidentalis* and the related *F. intonsa* (Hamilton *et al.*, 2005; Zhu *et al.*, 2012). Lavandulyl acetates are known components of sex pheromones in some insect species, such as *Planococcus ficus* (Zada *et al.*, 2003), but the role of (*R*)-lavandulyl acetate in *F. occidentalis* remains unknown. It was found to arrest females in laboratory experiments (Olaniran, 2013) and reduced trap catch when combined with neryl (*S*)-2-methylbutanoate at a 1:1 ratio when compared to neryl (*S*)-2-methylbutanoate alone in field experiments (Hamilton *et al.*, 2005), which suggests that it may not be part of the aggregation pheromone. However, Zhu *et al.* (2012) suggest that both compounds are part of the aggregation pheromone and that the ratio and doses of the two compounds plays an important role in interspecies recognition between *F. occidentalis* and *F. intonsa*, but they

did not test their theory with synthetic chemicals. The most frequent ratio of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate found in *F. occidentalis* has been about 5:1, but the ratios varied from about 3:1 to 47:1 (Kirk & Hamilton, 2004; Dublon *et al.*, 2008; Zhu *et al.*, 2012) (A. Sudhakhar, pers. comm., 2011), which suggests that the ratio is not critical to the response. (*R*)-lavandulyl acetate might be a synergist for neryl (*S*)-2-methylbutanoate at the right ratio or dose, or it might have a completely different role, in which case the ratio is likely to be variable and irrelevant to increasing trap catches.

Less volatile chemicals may also form part of aggregation pheromones at a shorter range. Some cuticular hydrocarbons are pheromones, for example, (*Z*)-9-tricosene is a sex pheromone attracting female houseflies (*Musca domestica*) (Carlson *et al.*, 1971). Others are synergistic, for example, a specific hydrocarbon causes the yellow peach moth (*Conogethes punctiferalis*) to spend more time in contact with, and in the vicinity of, a sex pheromone lure (Xiao *et al.*, 2012), which could translate into increased trap catch. Several cuticular hydrocarbons have been identified from samples of mixed sex *F. occidentalis* adults and larvae (Golebiowski *et al.*, 2007; Zhao *et al.*, 2011), but a contact hydrocarbon pheromone, 7-methyltricosane (Figure 5.1 E), is produced by adult males (Olaniran *et al.*, 2013). It is probably involved in species recognition and causes females to stay in the vicinity of the pheromone. In laboratory tests, there was only a contact response and the compound has low volatility (bp 411°C) (D. Hall, pers. comm., 2013) (Olaniran *et al.*, 2013), so it seems unlikely that the contact pheromone forms part of the aggregation pheromone, although it could improve trap catch by reducing escape rate. Two other male-produced compounds (9-methylpentacosane and 7-methylpentacosane) have been found in small quantities, but their role is unknown (Olaniran *et al.*, 2013). They could have a similar role to 7-methyltricosane in view of their similarity, as cuticular hydrocarbon effects often relate to groups of compounds. A pilot experiment was carried out to test whether 7-methyltricosane affects trap catch, but it is not reported here.

Plant volatiles and their analogues can influence the response to pheromone in phytophagous insects. In some species of Diptera and Coleoptera with male-produced pheromones, the response to pheromone is synergised by plant volatiles, which indicate feeding or oviposition sites. Synergism occurs when the interaction of two or more substances produces a combined effect greater than the sum (for untransformed data) or product (for log-transformed data) of their separate effects. For example, the response to aggregation pheromone in the mountain pine beetle (*Dendroctonus ponderosae*) is

synergised by monoterpenes from their host, pine bark (Borden *et al.*, 2008) and the response to aggregation pheromone in the fruit fly (*Drosophila virilis*) is synergised by the odour of willow bark, on which they feed and lay eggs (Landolt & Philips, 1997). The synergistic effect can be great, and the addition of fermented bread dough to aggregation pheromone increased trap catch by about a factor of 300 in *Carpophilus mutilatus* (Bartelt *et al.*, 1993a). In some species, such as *Cydia molesta*, specific mixtures of volatiles were attractive when the individual components were not (Natale *et al.* 2003), although testing multiple mixtures of plant volatiles was beyond the scope of this project. Some species, like the palm weevil (*Rhynchophorus palmarum*) only respond to pheromone in the presence of host odour (Rochat *et al.*, 1991). These strategies maximise the chance of meeting females that are visiting host plants to feed and lay eggs.

The polyphagous *F. occidentalis* is attracted to many floral scents and their analogues, which have been shown to increase trap catch (see Table 1.1). In this study, the plant volatile analogue methyl isonicotinate (Figure 5.1 F) was used to test whether there is a synergistic interaction between pheromone and plant volatiles, which could improve trap catch. It is known to be attractive to *F. occidentalis* (Davidson *et al.*, 2005) and is commercially available to growers (Lurem-TR, Koppert, Berkel en Rodenrijs, NL). In comparison studies, methyl isonicotinate gave an increase in trap catch of *F. occidentalis* similar to that of the aggregation pheromone (Broughton & Harrison, 2012). Interpretation of field responses are complicated by the fact that strawberry flowers contain the floral scents benzaldehyde and *p*-anisaldehyde (Hamilton-Kemp *et al.*, 1990), both of which are known attractants to *F. occidentalis* (Davidson *et al.*, 2008). In addition, there is increasing evidence of a strain of *F. occidentalis* in Spain that is not responding to methyl isonicotinate (Mette Nielsen, pers. comm., 2013). Full understanding of the interaction between pheromone and different floral scents would require extensive laboratory studies where competing scents were controlled, but in this study the objective was to improve trap catch in the field, so experiments were carried out in sweet pepper (Spain) and strawberry (UK) crops, where competing scents from flowers were present.

The design, colour and placement of a trap can affect the relative increase in trap catch with additional pheromone. A wide range of insect traps are available commercially and flat coloured sticky traps are most widely used for trapping *F. occidentalis* (Yudin *et al.*, 1987; Vernon & Gillespie, 1990, 1995; Brødsgaard, 1993b; Chen *et al.*, 2006). The colour and size of sticky traps have a great effect on *F. occidentalis* trap catch (see Chapters 1 and

6), but the effect of trap colour on pheromone trap catch is not known. A number of trap designs have been developed specifically for pheromone use in Lepidoptera as they change the plume shape to increase trap catch by aiding trail-following (Jones, 1998), although there is no evidence that *F. occidentalis* follow odour plumes. Systematic testing of trap types was beyond the scope of this study, but the delta trap (Figure 5.3 C) was tested as it is the most common pheromone trap design in commercial use.

A better understanding of thrips flight behaviour in response to different cues would help to interpret observed trap catches. The mating behaviour of *F. occidentalis* in the field was observed by Terry and Gardner (1990). Males defend a small territory (lek-like) in visible positions to attract females, such as the corolla of prominent white flowers or on attractively coloured surfaces (Terry & DeGrandi-Hoffman, 1988; Terry & Schneider, 1993). Typically females visit a swarm, mate and then leave (Terry & Gardner, 1990). Laboratory studies have identified several behaviours that could result in increased trap catch in the field. Some laboratory studies show increased thrips take-off in response to scent cues, followed by flight towards a visual stimulus once airborne (Brødsgaard, 1990; Smits *et al.*, 2000; van Tol *et al.*, 2012). Both methyl isonicotinate and neryl (S)-2-methylbutanoate increase the activity and take-off in *F. occidentalis* (van Tol *et al.*, 2012; Olaniran, 2013). Thrips move towards attractive scents in olfactometer experiments (Koschier *et al.*, 2000). Odours can also arrest thrips flight at certain concentrations and induce landing (Teulon *et al.*, 1999; Berry *et al.*, 2006) and Kirk (1985c) suggests that thrips could use a scent cue more efficiently as an arrestant or to stimulate a visual response because the cue could be used in still air. Teulon *et al.* (2007a) showed that an attractive odour only affected trap catch of *T. tabaci* over relatively short distances up to 10 m, but declining by 50% within 1.3 m from the odour source (methyl isonicotinate). If visual cues are dominant over scent for landing in *F. occidentalis* it could limit trap catch as the thrips may land on flowers before reaching a pheromone trap. Most flight occurs near or just above the top of the crop (Shipp & Zariffa, 1991; Mateus, 1998) where most flowers and new growth occurs and the attraction of the flowers (which often contain pheromone-producing thrips) may be greater than that of a pheromone trap. Laboratory experiments may not translate directly to the field as the response to odour changes according to various factors, such as hunger status of the insect (Davidson *et al.*, 2006), dose (Koschier *et al.*, 2000), host plant odours (Davidson *et al.*, 2009) and wind speed (see below).

Frankliniella occidentalis is not a strong flyer, which may limit its ability to fly upwind towards a trap. They have an estimated flight speed of 4-8 km h⁻¹ (Mateus, 1998) and land at wind-speeds above 8 km h⁻¹ in laboratory conditions (Teulon *et al.*, 1999). In the field, reduced flight has been observed at wind speeds above 15 km per h (Pearsall, 2002), although *F. occidentalis* can move up-wind by staying close to the ground and making small ‘hops’ and by timing their flights at times of the day when wind speeds are below 15 km per h (Ben-Yakir & Chen, 2008). Weak flight might be expected to limit trap catches, but some thrips species of a similar size and flying ability to *F. occidentalis*, such as *T. obscuratus* (and other small insects such as aphids), show consistently higher trap catches (sometimes by a factor >1000) in response to scents than *F. occidentalis* in the field (Teulon *et al.*, 1993; El-Sayed *et al.*, 2014). The most likely explanation for this is their flight behaviour. In New Zealand, *T. obscuratus* migrate annually from gorse bushes, where they overwinter, into stone fruit orchards during flowering and fruiting. Once temperatures are suitable, they launch themselves into the wind in large numbers in response to specific wind directions, then drop down in response to olfactory and visual cues (McLaren *et al.*, 2010). In contrast, *F. occidentalis* typically overwinters and builds up inside protected crops (see Chapter 3), so there is likely to be less flight than when thrips are migrating in from outside the crop and therefore less response to scented traps. Trap catches in outdoor crops are typically greater than in protected crops, but the increase in *F. occidentalis* trap catch with pheromone (compared to untreated controls) was similar ($\times 2$ - $\times 3$) in protected pepper (Gómez *et al.*, 2006), semi-protected strawberry (Sampson & Kirk, 2013) and in outdoor top fruit (Broughton & Harrison, 2012), so the trapping response does not relate to the level of protection alone. More information on *F. occidentalis* flight, the proportion of resident and immigrant thrips in these crops and on how that affects trap catch is required to fully interpret trap catches.

The overall aim of the experiments in this chapter was to determine whether the pheromone trap catch of *F. occidentalis* could be enhanced by specific dose rates, chiral forms or ratios of the male-produced compounds and whether response to the pheromone could be improved by trap colour, trap type, trap placement or plant volatiles. Specific aims were to:

- (1) test whether the dose rate of neryl (*S*)-2-methylbutanoate affects the trap catch;
- (2) test whether the chirality of neryl (*S*)-2-methylbutanoate is critical to the trap catch;
- (3) test whether the ratio of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate affects trap catch compared to neryl (*S*)-2-methylbutanoate alone;
- (4) test whether the dose of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate at a 5:1 ratio affects trap catch;
- (5) confirm which chiral form of lavandulyl acetate is biologically active;
- (6) test whether pheromone trap catch is enhanced by specific trap colours and whether there is an interaction between the responses to colour and scent;
- (7) test whether the height of the trap above the crop affects pheromone trap catch;
- (8) test whether pheromone released from treatment traps is reaching control traps and boosting their catch;
- (9) test whether the pheromone trap catch is synergised by methyl isonicotinate in pepper and strawberry crops.

5.2. Materials and Methods

A series of experiments was carried out in two commercial pepper crops grown in multispan plastic houses in the Murcia region of Spain (Table 5.1, Figure 5.2) during April 2011. The thrips population at both sites consisted of > 97% *F. occidentalis* (see 5.3.9) and there were few other pests, which allowed for rapid screening of the different pheromone components without contamination of traps with other insect species. The plastic houses were selected for their infestation level, size and ease of access to traps. Within both houses there were local gradients in thrips density, with more thrips in the warmer central area of the houses and fewer around the perimeter. The experiments were placed centrally in the houses and the experimental design was blocked to reduce these effects, however there was still much variation that could not be removed statistically. As a result there was considerable background variation that would tend to obscure real effects unless the experiments were well replicated. The following methods were common to most of the experiments carried out in Spanish pepper crops in this chapter and will not be repeated in each section. Further details that are specific to individual experiments are included within the chapter.

Blue (Takitrapline b, Syngenta Bioline Ltd, Clacton, UK) or yellow sticky traps (Takitrapline y, Syngenta Bioline Ltd) were used unless stated otherwise. These were 10 cm wide by 25 cm tall and are widely used for monitoring *F. occidentalis*. Yellow traps were used to test the pheromone components as they are less attractive than blue traps so more thrips were available to respond to the scents. They were also used for the practical reason that the thrips and their features are easier to see on a yellow trap than on a blue trap. Experiments were laid out in randomised complete block designs with appropriate controls. In most experiments the traps within a block were spaced 4.8 m (4 plants) apart along a single row (Figure 5.3 A), which reflects the planting distance of 1.2 m between plants within a row. It is possible that some scent from treated traps reached control traps (Teulon *et al.*, 2007a) but similar spacing has detected differences in previous experiments (Hamilton *et al.*, 2005) and wider spacing would have placed some traps in areas with very few thrips by extending the overall size of the experiment. Each block was along a different plant row and separated by either 6 plant rows (6 m) or 9 plant rows (9 m) according to the size of the greenhouse. Traps were suspended vertically (portrait orientation) with the base of the trap about 10 cm above crop height (unless stated otherwise) by attaching them with wooden clothes pegs to vertical strings supporting the crop (Figure 5.3 A).

The source of the different pheromone and plant volatile analogue components used (below) apply to all the chemicals referred to subsequently in this chapter and will not be repeated in each section.

Neryl (*S*)-2-methylbutanoate was synthesised by Prof. David Hall and Mr Dudley Farman at NRI (Natural Resources International, Chatham, Kent, UK) from (*S*)-2-methylbutanoic acid (SigmaAldrich, Gillingham, Dorset, UK) as described by Hamilton *et al.* (Hamilton *et al.*, 2005) and had enantiomeric excess (ee) of 97.8% as determined by gas chromatographic analysis on a chiral cyclodextrin column. Neryl (*R*)-2-methylbutanoate was synthesised by NRI similarly from (*R*)-2-methylbutanoic acid provided by Dr Aijun Zhang (USDA, Beltsville) (Zhang *et al.*, 2004) and had ee of 99.0%. Both compounds were at least 98% pure by GC analysis.

The enantiomers of lavandulyl acetate were synthesised by NRI by resolution of lavandulol (SigmaAldrich) with porcine pancreatic lipase (SigmaAldrich) and vinyl acetate in petroleum ether with separation of alcohol and acetate by silica gel chromatography.

Four cycles gave (*R*)-lavandulyl acetate with ee 97.2% and (*S*)-lavandulyl acetate with ee 86.8%, both compounds being at least 98% pure by GC analysis.

Pheromone dispensers were either polyethylene vials (0.5 ml, 9 mm diam. × 23 mm long, 1.5 mm thick; Just Plastics, Norfolk, UK) or natural rubber septa (6.3 mm diam. × 10.8 mm long; International Pheromone Systems Ltd., Cheshire, UK), as used for the commercial lures (Thripline_{ams}, Syngenta Bioline Ltd, Clacton, UK). Pheromones were dissolved in petroleum: ether (bp 40-60°C; Fisher Scientific) or *n*-hexane (>97% purity, Merck, Germany) and added to the dispenser in a volume of 0.1 ml. After evaporation of the solvent the lid was closed on the vials.

Methyl isonicotinate (250 µl) (98%, SigmaAldrich) was dispensed from polyethylene sachets (50 mm x 50 mm x 120 µ thick) similar to those used in the commercial product Lurem (Koppert). These were prepared by heat sealing layflat tubing (Transatlantic Plastics, Southampton, UK) and gave a release rate of 12.8 mg per day at 22°C.

Lures containing specific volumes of the pheromone and plant volatile components were largely made up in the UK by Prof. Gordon Hamilton or by Prof. David Hall and Mr Dudley Farman (NRI) before travelling to Spain and were packed separately in aluminium foil wrappers to prevent contamination. Some lures were made up *in situ* by Prof. Hamilton and some experiments used commercially available lures (Thripline_{ams}, Syngenta Bioline Ltd, Clacton, UK).

Release rate measurements made at NRI (27°C and 8 km h⁻¹ windspeed) showed that lavandulyl acetate was released very rapidly from septa, essentially within 5 days, and release of neryl (*S*)-2-methylbutanoate declined exponentially over about 30 days. Release of both compounds from vials was more uniform with release of neryl (*S*)-2-methylbutanoate about 30% of the initial release rate from septa. Thus a dose of 100 µg neryl (*S*)-2-methylbutanoate in vials gave the same release rate as 30 µg neryl (*S*)-2-methylbutanoate from rubber septa (the amount used in commercial lures by Syngenta Bioline Ltd), and this was calculated to be about 0.4 µg per day at 27°C. As (*R*)-lavandulyl acetate (bp 229°C) is more volatile than neryl (*S*)-2-methylbutanoate (bp 113°C), half the volume of (*R*)-lavandulyl acetate was required to give the same release rate as neryl (*S*)-2-methylbutanoate (D. Hall, pers. comm., 2011). Before the start of the experiments, the dispensers (vials containing 100 µg and septa containing 30 µg neryl (*S*)-2-methylbutanoate) were tested in the field and both dispensers increased trap catch by

about the same proportion ($\times 1.3$) so it was concluded that vials could be used in further experiments (Sampson, unpublished data, 2011).

In the majority of experiments, a single polyethylene vial (Just Plastics) or a single natural rubber septum (International Pheromone Systems Ltd.) was placed vertically in the middle of the slightly concave side of the dry traps (Figure 5.3 B). The sticky glue on the trap was usually sufficient to hold the lures in place during the experiments, although lures occasionally fell off and affected blocks were omitted from the analysis. Dedicated latex gloves were used to handle lures for each treatment to prevent cross-contamination; these were disposed of after each experiment. The sachets and their controls were placed at the tops of the traps and held in place with wooden clothes pegs, which were disposed of after a single use. All traps were oriented so that the septum/vial/sachet side faced north to avoid direct sunlight on the release device. However, some traps twisted during the experiment, so that the initial orientation was not always maintained. Maximum and minimum temperature and humidity were recorded during each experiment with a digital thermo-hygrometer (Thermo-Hydro, RS 212-124, Oregon Scientific, Northants, UK), which was strapped to a polytunnel post at crop height and spaced so that it did not touch the metal post. Traps were removed from the crop after 24 hours, wrapped individually in polythene and stored in a freezer.

Trap catches of *F. occidentalis* were counted under a stereoscopic microscope (Wild AG, Heerbrugg) in the Keele laboratory, using the methods described in Chapter 2. Aeolothripid (with broad wing fringes) and phlaeothripid thrips (with elongated last abdominal segment) were excluded from the counts. The great majority of the thrips on traps were *F. occidentalis* and the key identification features detailed in Chapter 2 were usually visible under the microscope without mounting the thrips on slides. Occasional individuals of other species were present (typically 1-5 per trap). To confirm the identifications, one thrips was randomly selected, using an acetate sheet with a 0.5 cm^2 grid marked on it and random numbers, from 100 randomly selected control traps (from all experiments) per site ($n = 200$ thrips). These thrips were removed from the traps, mounted on slides and identified to species, using the methods detailed in Chapter 2.

All experiments in Spain were carried out jointly by me, Dr William Kirk and Prof. Gordon Hamilton. Experimental design was discussed and agreed between us and was informed by previous experiments (Hamilton *et al.*, 2005). Some rapid counting of thrips

on traps was carried out by Prof. Gordon Hamilton and me in the field to check that the pheromone was active, but all thrips were re-counted and sexed by me under a binocular microscope in the laboratory. Following initial data analysis in Spain carried out by me and Dr William Kirk, all data were re-analysed by me once the final counts had been made back at Keele.

One experiment in this chapter was carried out in UK strawberry. It was a repeat of an experiment carried out in Spain using plant volatiles where there was some doubt about the response of Spanish *F. occidentalis* populations to the scent. The experiment was designed, carried out and analysed by me and the methods are detailed separately within the chapter.

5.2.1. Does the release rate of neryl (*S*)-2-methylbutanoate affect trap catch?

To test the effect of release rate of neryl (*S*)-2-methylbutanoate on trap catch, lures containing different doses of the aggregation pheromone were added to traps that were hung above a crop and the trap catch compared between doses after 24 h. If the release rate is not critical then a higher dose can be used in commercial lures, which can last longer in the field. The response of *F. occidentalis* to neryl (*S*)-2-methylbutanoate release rate was tested in a commercial pepper crop (Site 1, Table 5.1, Figure 5.2 A) using yellow sticky traps (Takitrapline y) laid out in a randomised complete block design. There were 20 blocks and one replicate per block with 4.8 m between traps within a block and 6 m between blocks. One trap dropped during the experiment, so the affected block was omitted. Treatments included four release rates of neryl (*S*)-2-methylbutanoate and a control, with one vial containing the treatments was placed centrally on the front of each trap. Treatments were:

- 10 µg neryl (*S*)-2-methylbutanoate dissolved in 100 µl solvent (a release rate of about 0.04 µg per day);
- 100 µg neryl (*S*)-2-methylbutanoate dissolved in 100 µl solvent (a release rate of about 0.4 µg per day);
- 1000 µg neryl (*S*)-2-methylbutanoate dissolved in 100 µl solvent (a release rate of about 4 µg per day);
- 2000 µg neryl (*S*)-2-methylbutanoate dissolved in 100 µl solvent (a release rate of about 8 µg per day);
- 100 µl solvent only (control).

5.2.2. Does the chiral form of neryl -2-methylbutanoate affect trap catch?

Industrial sources of neryl (*S*)-2-methylbutanoate are usually contaminated with small amounts of the (*R*) form (e.g. neryl (*S*)-2-methylbutanoate from Merck, Germany, with 98% enantiomeric excess contains 1% of the (*R*) form). To test whether the chiral form of neryl 2-methylbutanoate is critical to trap catch, lures containing different ratios of the two enantiomers and an untreated control were added to traps that were hung above a crop and the trap catch compared after 24 h. If the chirality is critical to the response then a pure source of the pheromone is required to maximise trap catch. The response of *F. occidentalis* to the different enantiomeric forms of neryl 2-methylbutanoate (Figure 5.1 A, B) was tested in a commercial pepper crop (Site 2, Table 5.1, Figure 5.2 B) using yellow sticky traps (Takitrapline y) laid out in a randomised complete block design. There were 20 blocks and one replicate per block with 4.8 m between traps within a block and 9 m between blocks. Treatments included neryl (*S*)-2-methylbutanoate, neryl (*R*)-2-methylbutanoate, a racemic mix of both enantiomers and a control, with one vial containing the treatments placed centrally on the front of each trap. The vials were made up by NRI to give a release rate of 0.4 µg per day (D. Hall, pers. comm., 2011) and the treatments were:

- 100 µg neryl (*S*)-2-methylbutanoate dissolved in 100 µl solvent;
- 100 µg neryl (*R*)-2-methylbutanoate dissolved in 100 µl solvent;
- 100 µg neryl (*S*)-2-methylbutanoate plus 100 µg neryl (*R*)-2-methylbutanoate dissolved in 100 µl solvent;
- 100 µl solvent only (control).

If the purity of neryl (*S*)-2-methylbutanoate is critical to the response, then the addition of a small amount more of the (*R*) form will affect trap catch. To test this, the experiment above was repeated at the same site (Site 2), using the same trap type (Takitrapline y) and trap spacing (4.8 m) as above, but with a smaller dose of the (*R*) form. Rubber septa were used instead of vials, which were prepared *in situ* by Prof. G Hamilton whilst in Spain. The treatments were:

- 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl solvent (about 1% (*R*));
- 30 µg neryl (*S*)-2-methylbutanoate plus 1 µg neryl (*R*)-2-methylbutanoate; dissolved in 30 µl solvent (about 4% (*R*));
- 30 µl solvent only (control).

5.2.3. Does lavandulyl acetate increase trap catch?

Lavandulyl acetate could be part of the *F. occidentalis* pheromone at a specific chiral form, dose, or ratio to neryl (*S*)-2-methylbutanoate, but it may have a different role altogether (see 5.1). If no increase in trap catch can be identified with the addition of lavandulyl acetate at different ratios, chiral forms and doses, then it is further evidence that it is not part of the aggregation pheromone and so there would be no benefit in adding it to the aggregation pheromone lures to increase trap catch.

5.2.3.1. Does the ratio of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate affect trap catch?

The response of *F. occidentalis* to different ratios of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate was tested in a commercial pepper crop (Site 2, Table 5.1, Figure 5.2 B) using yellow sticky traps (Takitrapline y) laid out in a randomised complete block design. There were 20 blocks and one replicate per block with 4.8 m between traps within a block and 9 m between blocks. Treatments covered the full range of ratios observed in *F. occidentalis* (see 5.1). One vial containing the treatments was placed centrally on the front of each trap. The vials were made up by NRI and were calibrated to give the specified release rate, rather than a ratio based on volume of pheromone. The release rate of 100 neryl (*S*)-2-methylbutanoate was the same as that of 50 µg (*R*)-lavandulyl acetate, which was 0.4 µg per day. The treatments were:

- 100 µg neryl (*S*)-2-methylbutanoate dissolved in 100 µl solvent (release ratio of 1:0 neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate);
- 100 µg neryl (*S*)-2-methylbutanoate plus 50 µg (*R*)-lavandulyl acetate dissolved in 100 µl solvent (release ratio of 1:1);
- 100 µg neryl (*S*)-2-methylbutanoate plus 10 µg (*R*)-lavandulyl acetate dissolved in 100 µl solvent (release ratio of 5:1);
- 100 µg neryl (*S*)-2-methylbutanoate plus 5 µg (*R*)-lavandulyl acetate dissolved in 100 µl solvent (release ratio of 10:1);
- 100 µg neryl (*S*)-2-methylbutanoate plus 1 µg (*R*)-lavandulyl acetate dissolved in 100 µl solvent (release ratio of 50:1);
- 100 µl solvent only (control).

5.2.3.2. Does the release rate of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate (5:1) affect trap catch?

The response of *F. occidentalis* to low, medium and high release rates of a 5:1 ratio of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate was tested in a commercial pepper crop (Site 1, Table 5.1, Figure 5.2 A) using yellow sticky traps (Takitrapline y) laid out in a randomised complete block design. A 5:1 ratio of release rates of the compounds was used, as this is the most frequent ratio found in *F. occidentalis* (see 5.1) (A. Sudhakar, pers. comm., 2011). There were 20 blocks and one replicate per block with 4.8 m between traps within a block and 6 m between blocks. One vial containing the treatments was placed centrally on the front of each trap. The vials were made up by NRI and were calibrated to give the specified doses at a release rate ratio of 5:1 (D. Hall, pers. comm., 2011). Treatments were:

- 10 µg neryl (*S*)-2-methylbutanoate plus 1 µg (*R*)-lavandulyl acetate dissolved in 100 µl solvent (low release rate of 0.04 µg neryl plus 0.008 µg lavandulyl per d);
- 100 µg neryl (*S*)-2-methylbutanoate plus 10 µg (*R*)-lavandulyl acetate dissolved in 100 µl solvent (medium release rate of 0.4 µg neryl plus 0.08 µg lavandulyl per d);
- 1000 µg neryl (*S*)-2-methylbutanoate plus 100 µg (*R*)-lavandulyl acetate dissolved in 100 µl solvent (high release rate of 4 µg neryl plus 0.8 µg lavandulyl per d);
- 100 µl solvent only (control).

5.2.3.3. Does the chiral form of lavandulyl acetate affect trap catch?

The response of *F. occidentalis* to the two chiral forms of lavandulyl acetate (Figure 5.1 C, D) was tested in a commercial pepper crop (Site 2, Table 5.1, Figure 5.2 B) using yellow sticky traps (Takitrapline y) laid out in a randomised complete block design. There were 20 blocks and one replicate per block with 4.8 m between traps within a block and 9 m between blocks. Treatments included (*R*)-lavandulyl acetate, (*S*)-lavandulyl acetate, a racemic mix of equal amounts of both enantiomers and a control, with one vial stuck centrally on the front of each trap. The release rate of 50 µg lavandulyl acetate was about 0.4 µg per day. The vials were made up by NRI and treatments were:

- 50 µg (*R*)-lavandulyl acetate dissolved in 100 µl solvent;
- 50 µg (*S*)-lavandulyl acetate dissolved in 100 µl solvent;
- 50 µg (*R*)-lavandulyl acetate plus 50 µg (*S*)-lavandulyl acetate dissolved in 100 µl solvent;
- 100 µl solvent only (control).

5.2.4. Is the pheromone trap catch enhanced by certain trap colours?

The effect of trap colour on the response of *F. occidentalis* to neryl (*S*)-2-methylbutanoate was tested in a commercial pepper crop (Site 1, Table 5.1, Figure 5.2 A) using yellow, blue, clear and black sticky traps with a black grid on the back (10 cm by 25 cm, Impact trap, Russell IPM, Deeside, UK), laid out in a randomised complete block design. Blue and yellow traps were selected as they are attractive to *F. occidentalis* and widely used for monitoring pests in greenhouses. Clear and black traps were selected as they are less visibly attractive. There were 20 blocks and one replicate per block with 3.6 m between traps within a block and 6 m between blocks. The distance between traps was reduced from every four plants (4.8 m) to every three plants (3.6 m) so that the eight treatments within a block could be fitted down a single plant row while avoiding the edges of the greenhouse. One trap dropped during the experiment, so the affected block was omitted from the analysis. Treatments were the four different colours of sticky trap, each with or without the *F. occidentalis* aggregation pheromone, neryl (*S*)-2-methylbutanoate. Rubber septa were used for the pheromone and control treatments, which were placed centrally on the traps. The septum was treated with 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl hexane and the control septum with 30 µl hexane only.

5.2.5. Is the pheromone trap catch enhanced by trap type?

Some traps, such as delta traps (Figure 5.3 C), are designed to enhance the pheromone plume, which increases trap catch in species (e.g. moths) that follow scent trails (see 5.1). The response of *F. occidentalis* to neryl (*S*)-2-methylbutanoate was tested on sticky monitoring traps and in delta traps, in a commercial pepper crop (Site 2, Table 5.1, Figure 5.2 B) laid out in a randomised complete block design. The design should have compared sticky and delta traps of the same colour and surface area, but none were available at the time, so different coloured traps were used. Although this confounds the results between trap type and trap colour, it was sufficient to test whether a large increase in pheromone

trap catch would result from the delta trap design, which could be investigated further. There were 20 blocks and one replicate per block with 4.8 m between traps within a block and 9 m between blocks. Treatments included blue sticky traps (Takitrapline b) and brown cardboard delta traps (273 mm length, 130 mm height, æcos, Kimpton, UK) with a blue sticky monitoring trap insert, with and without neryl (*S*)-2-methylbutanoate, with one septum placed centrally on the front of the sticky monitoring trap or on the middle (upper surface) of each delta trap insert in the delta trap. The pheromone septum was impregnated with 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl solvent and the control septum with 30 µl solvent only. Trap counts were compared using half of the trap count of the sticky monitoring traps (i.e. the total trap count divided by two), so that the trap area was equivalent to the one side of trap exposed inside the delta trap.

5.2.6. Does trap height above the crop affect pheromone trap catch?

The response to pheromone was compared at two different heights above the crop to test whether pheromone can attract thrips away from the crop. If so, the ratio of treatment catch to control catch would be expected to be at least as good between the higher traps as between the lower traps. If the higher traps are less effective than lower traps, then traps would need to be sited close to the crop for maximum trap catch. The response of *F. occidentalis* to neryl (*S*)-2-methylbutanoate at different trap heights was tested in a commercial pepper crop (Site 2, Table 5.1, Figure 5.2 B) using yellow sticky traps (Takitrapline y) laid out in a randomised complete block design. There were 20 blocks and one replicate per block with 3.6 m between traps within a block and 6 m between blocks. Treatments included trap heights with the base of the trap 20 cm or 45 cm above the crop canopy, with and without neryl (*S*)-2-methylbutanoate, with one septum placed centrally on the front of each trap. A trap height of 20 cm rather than the usual 10 cm was used because the crop had wilted overnight and the crop recovered to about 10 cm below the trap during the experiment. The pheromone septum was impregnated with 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl solvent and the control septum with 30 µl solvent only.

5.2.7. Does trap spacing affect pheromone trap catch?

The response to pheromone was compared at two different spacings between pheromone and control traps to test whether pheromone released from pheromone traps was reaching control traps and boosting their catch. If so, the ratio of treatment catch to

control catch would be expected to be higher on traps spaced far apart and lower on traps spaced close together. Control traps far apart from treatment traps (far control) would be expected to have lower trap catches than all the other trap types, because they are least likely to have pheromone reaching them from surrounding traps. The response of *F. occidentalis* to neryl (*S*)-2-methylbutanoate at different trap spacing was tested in a commercial pepper crop (Site 1, Table 5.1, Figure 5.2 A) using blue sticky traps (Takitrapline b) laid out in a randomised complete block design. There were 20 blocks and one replicate per block with 6 m between blocks. Within each block there were two pairs of traps spaced at 1.2 m and 6 m and the position of each treatment within a pair was chosen randomly. The treatments within each pair were with and without neryl (*S*)-2-methylbutanoate, with one septum placed centrally on the front of each trap. The pheromone septum was filled with 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl solvent and the control septum with 30 µl solvent only.

5.2.8. Is response to pheromone synergised by plant volatiles?

Plant volatiles and their analogues are known to synergise the response to aggregation and sex pheromones in some insect species (see 5.1) and can increase thrips trap catch on their own (see Table 1.1). Traps baited with the different scents, combined scents or no scents were placed in pepper and strawberry crops and the trap catches compared. If a synergistic or even additive increase in trap catch is found, then there would be a benefit in combining the scents to increase trap catch.

5.2.8.1. In a pepper crop in Spain

The effect of a plant volatile on response to pheromone was tested in a commercial pepper crop (Site 1, Table 5.1, Figure 5.2 A) using yellow sticky traps (Takitrapline y) laid out in a randomised complete block design. There were 20 blocks and one replicate per block with 4.8 m between traps within a block and 6 m between blocks. On each trap one septum and one sachet were placed side by side, 2 cm from the top, on the front (north facing side) of each trap. The sachets were made up by NRI. The pheromone septum was loaded with 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl solvent, the methyl isonicotinate sachet contained 250 µl neat solution, the control septum contained 30 µl solvent only and the control sachet was an empty sachet. The release rate for the different treatments matched those of the commercial products (Thripline_{ams}, Syngenta Bioline Ltd, Clacton, UK and Lurem-TR, Koppert, Rodenrijs, NL). The methyl isonicotinate was

placed in a sachet rather than a septum because of the large volume of liquid required. The methyl isonicotinate sachets had a release rate of about 12.8 mg per day and the neryl (*S*)-2-methylbutanoate had a release rate of about 0.4 µg per day (D. Hall, pers. comm., 2011), i.e. the release rate of the plant volatile analogue was $\times 32,000$ that of the aggregation pheromone. Treatments were:

- 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl solvent in a septum with a blank sachet;
- 250 µl methyl isonicotinate in a sachet with a septum containing 30 µl solvent only;
- 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl solvent in a septum and 250 µl methyl isonicotinate in a sachet;
- a septum containing 30 µl solvent only and a blank sachet (control).

5.2.8.2. *In a strawberry crop in the UK*

The effect of a plant volatile on response to pheromone was tested in a commercial semi-protected strawberry crop (cv. Finesse) near Tamworth, UK (Field 7, Table 2.1) using blue sticky traps (Impact trap) laid out in a randomised complete block design on 10 August 2012. Blue traps were used because blue is more likely to be used commercially for mass trapping. Traps were placed between the strawberry beds in the leg area of the polytunnel so that they were out of the way of crop workers. They were stuck vertically (landscape orientation) onto bamboo canes (60 cm long), which were pushed into the soil so that the bottom edge of the traps were about 10 cm above the height of the crop, which was about 40 cm high (Figure 5.3 D). There were 18 blocks and one replicate per block with 6.6 m (three posts) between traps within a block and 6.5 m (one tunnel width) between blocks. Treatments were:

- 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl solvent in a septum with a blank sachet;
- 250 µl methyl isonicotinate in a sachet with a septum containing 30 µl solvent only;
- 30 µg neryl (*S*)-2-methylbutanoate dissolved in 30 µl solvent in a septum and 250 µl methyl isonicotinate in a sachet;
- a septum containing 30 µl solvent only and a blank sachet (control).

On each trap one septum and one sachet were placed side by side, 2 cm from the top, on the front (north facing side) of each trap (Figure 5.3 D). The methyl isonicotinate sachets contained 250 μ l neat solution and were made up by NRI. Commercial lures were used for the pheromone septum (Thripline_{ams}). The control septum was filled with 30 μ l solvent only and the control sachet was an empty sachet. The traps were removed after 8 h in the crop (10.30 h to 18.30 h), wrapped separately in polythene and stored in a freezer.

Trap catches of *F. occidentalis* were counted under a stereoscopic microscope (Wild AG, Heerbrugg) in the Keele laboratory, using the methods described in Chapter 2. The great majority of the thrips on traps appeared to be *F. occidentalis* and one thrips was selected at random from each control trap and identified to species to confirm the identification, using the methods detailed in Chapter 2 ($n = 18$ thrips).

To determine the thrips species and density of thrips in the crop, ten plants were selected arbitrarily from the experimental field and the number of flowers per plant was counted and one medium-aged strawberry flower (see Chapter 2) was picked from each of the sample plants ($n = 10$ flowers). The flowers were pooled and placed in 70% alcohol so that adult thrips could be extracted. One hundred adult thrips were selected at random from the alcohol sample and identified to species using the methods detailed in Chapter 2 ($n = 100$ thrips).

5.2.9. Statistical analysis

Statistical analysis was carried out using Minitab 16 (Minitab Incorporated, Pennsylvania, USA). Data and residuals were checked for normality using an Anderson-Darling test. Parametric analysis of variance was used on $\log_{10}(n+1)$ transformed data to homogenise the variance. Multiple comparisons used Tukey's test. Where data were not normally distributed, Mann Whitney non-parametric tests were used for pair-wise comparisons. Data were considered statistically significant where $P < 0.05$.

In the trap colour experiment (see 5.2.4), the variances of thrips numbers on the more attractive trap colours were greater than those of the less attractive colours, reflecting the differences in their means. An assumption of analysis of variance is homogeneity of variance. Despite differences in variance between thrips numbers on different trap colours, the ratio of the largest cell variance to the smallest (F_{\max}) was less than 10, so analysis of variance was still considered appropriate (Tabachnick & Fidell, 2001). The residuals were normally distributed. Pair-wise comparisons between pheromone and control traps of each

trap colour used Tukey's test. The ratio of trap catch between each treatment and the control was calculated by comparing the untransformed trap catch on the two trap types within each block, and these ratios were compared using regression analysis between the relative effectiveness of pheromone (ratio of treatment: control catch) and the attractiveness of the trap colour (control catch for each colour).

Tables and figures show untransformed means to aid interpretation and allow more intuitive comparison with counts that would be used by growers, whilst statistical analysis used transformed data. The factor of increase or decrease in trap catch was calculated by dividing the treatment trap catch by the control trap catch using untransformed means.

5.3. Results

5.3.1. Does the release rate of neryl (*S*)-2-methylbutanoate affect trap catch?

Pheromone trap catch was not affected by the release rate of the pheromone over a wide range between 0.04 µg and 8 µg per day (Figure 5.4 A, B, Tukey's test, $P < 0.05$). There was a significant difference in trap catch between treatments overall, for females (ANOVA, $F_{(4, 72)} = 13.4$, $P < 0.001$) and males (ANOVA, $F_{(4, 72)} = 4.9$, $P = 0.002$). All the release rates of the aggregation pheromone, neryl (*S*)-2-methylbutanoate, increased trap catch compared to untreated control traps, by $\times 1.6$ -2.2 for females and by $\times 1.6$ -1.8 for males.

5.3.2. Does the chiral form of neryl -2-methylbutanoate affect trap catch?

For females, there was a significant difference in trap catch between the treatments overall (ANOVA, $F_{(3, 57)} = 6.7$, $P < 0.001$). The aggregation pheromone, neryl (*S*)-2-methylbutanoate, increased trap catch by $\times 1.8$, but there was no increase with neryl (*R*)-2-methylbutanoate or when the two chiral forms were combined in equal proportions (Figure 5.5 A). Analysis of treatment effects of the chiral forms for females was repeated using two-factor ANOVA with contrasts, to take account of the pheromone components in the mixed treatment. This showed a significant increase in trap catch with neryl (*S*)-2-methylbutanoate ($F_{(1, 57)} = 35.0$, $P < 0.001$) and a significant decrease with neryl (*R*)-2-methylbutanoate ($F_{(1, 57)} = 23.7$, $P < 0.001$) with no significant interaction between the two chiral forms ($F_{(1, 57)} = 2.4$, $P = 0.13$). For males there were no significant differences

between the treatments overall (ANOVA, $F_{(3, 57)} = 2.6$, $P = 0.065$), although the trends in the data were the same as for females (Figure 5.5 B) and a two-factor ANOVA with contrasts showed an increase in trap catch with neryl (*S*)-2-methylbutanoate and decrease with neryl (*R*)-2-methylbutanoate that were close to significant ($F_{(1, 57)} = 3.8$, $P = 0.056$ for (*S*); $F_{(1, 57)} = 2.8$, $P = 0.10$ for (*R*); $F_{(1, 57)} = 1.1$, $P = 0.31$ for interaction).

If purity of the neryl (*S*)-2-methylbutanoate is critical to the response, then a decrease in trap catch might be observed when only a small amount of the (*R*) form is added. When a small amount (1 µg, or about 4%) of neryl (*R*)-2-methylbutanoate was added to the 1% already present the pheromone reduced trap catch of both females and males by a small amount (about 4%) that was not significantly different from the trap catch neryl (*S*)-2-methylbutanoate alone (Figure 5.6 A, B, Tukey's test, $P < 0.05$). There was a significant difference in trap catch between treatments overall, for females (ANOVA, $F_{(2, 38)} = 19.0$, $P < 0.001$) and males (ANOVA, $F_{(2, 38)} = 4.1$, $P = 0.026$). Neryl (*S*)-2-methylbutanoate alone increased trap catch of females by $\times 1.5$ and males by $\times 1.3$. The trends were consistent with the experiment above.

5.3.3. Does lavandulyl acetate affect trap catch?

5.3.3.1. Does the ratio of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate affect trap catch?

The addition of (*R*)-lavandulyl acetate to the aggregation pheromone reduced the trap catch, with a trend towards decreasing trap catch with increasing proportion of (*R*)-lavandulyl acetate (Figure 5.7 A, B, Tukey's tests, $P < 0.05$). There was a significant difference in trap catch between treatments overall for females (ANOVA, $F_{(5, 95)} = 11.0$, $P < 0.001$), but not males (ANOVA, $F_{(5, 95)} = 1.6$, $P = 0.19$), although the trends in the data were similar for both sexes. The aggregation pheromone, neryl (*S*)-2-methylbutanoate, increased trap catch of females by $\times 2.0$ and of males by $\times 1.6$.

5.3.3.2. Does the release rate of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate (5:1) affect trap catch?

There was a significant effect of a 5:1 ratio of neryl (*S*)-2-methylbutanoate: (*R*)-lavandulyl acetate release rate on trap catch. There was an increase in trap catch of females at the higher release rates ($\times 1.8$ - 1.9) but not at the lower release rate (Figure 5.8 A, Tukey's test, $P < 0.05$), and the increases were not as high ($\times 0.6$) as those seen with the

same release rates of neryl (*S*)-2-methylbutanoate alone ($\times 2.1$ - 2.2 , Figure 5.4 A). None of the doses tested revealed a response specific to concentration over and above that expected from neryl (*S*)-2-methylbutanoate alone. There was a similar trend for males although the increases in trap catch with the two compounds were lower than for females ($\times 1.2$ - 1.4) and were not significantly different from the control (Figure 5.8 B, Tukey's test, $P < 0.05$). There was a significant difference in trap catch between treatments overall for females (ANOVA, $F_{(3, 57)} = 21.6$, $P < 0.001$), but not males (ANOVA, $F_{(3, 57)} = 1.1$, $P = 0.38$).

5.3.3.3. Does the chiral form of lavandulyl acetate affect trap catch?

(*R*)-lavandulyl acetate, and the two chiral forms combined, significantly reduced trap catch of females, whereas the enantiomer (*S*)-lavandulyl acetate had no effect (Figure 5.9 A, Tukey's test, $P < 0.05$). The treatment trends were the same in males, although the differences were not significant (Figure 5.9 B, Tukey's test, $P < 0.05$). Analysis of treatment effects of the chiral forms was repeated using two-factor ANOVA with contrasts, to take account of the components in the mixed treatment. This showed a significant reduction in trap catch with (*R*)-lavandulyl acetate for both sexes (females, $\times 0.63$, $F_{(1, 57)} = 23.4$, $P < 0.001$; males $\times 0.81$, $F_{(1, 57)} = 4.1$, $P = 0.047$) while (*S*)-lavandulyl acetate had no effect (females, $F_{(1, 57)} = 3.4$, $P = 0.07$; males, $F_{(1, 57)} = 0.5$, $P = 0.54$), and there was no interaction between the two chiral forms (females, $F_{(1, 57)} = 0.6$, $P = 0.46$; males, $F_{(1, 57)} = 1.0$, $P = 0.33$). There was a significant difference in trap catch between treatments overall for females (ANOVA, $F_{(3, 57)} = 9.1$, $P < 0.001$), but not for males (ANOVA, $F_{(3, 57)} = 1.8$, $P = 0.15$).

These results are consistent with previous experiments, showing that no increase in trap catch was identified with any of the ratios, doses or chiral forms of lavandulyl acetate tested. (*R*)-lavandulyl acetate reduced trap catches, especially of females, at a very low release rates, indicating that the thrips are very sensitive to it.

5.3.4. Is the pheromone trap catch enhanced by certain trap colours?

There was a strong effect of trap colour on trap catch of *F. occidentalis* (ANOVA, $F_{(3, 133)} = 175.0$, $P < 0.001$). Blue traps caught significantly more thrips than yellow traps ($\times 2.4$), clear traps ($\times 9.3$) or black traps ($\times 34.7$) (Figure 5.10). The aggregation pheromone increased trap catch (ANOVA, $F_{(1, 133)} = 16.0$, $P < 0.001$) in inverse relationship to the attractiveness of the trap colour (blue $\times 1.3$, yellow $\times 1.7$, clear $\times 1.9$, black $\times 3.4$). A general

linear model regression showed that there was a negative association between the relative effectiveness of pheromone (ratio of treatment: control catch) and the attractiveness of the trap colour (control catch for each colour) (Regression, $F_{(1, 55)} = 7.2$, $P = 0.01$).

5.3.5. Is the pheromone trap catch enhanced by trap type?

Blue sticky traps caught $\times 29$ more adult thrips than delta traps (Mann Whitney, $W_{19} = 820$, $P < 0.001$). The delta traps, which were brown with a blue sticky trap insert, caught very few thrips with or without pheromones, confirming the importance of the visual component of the traps for absolute trap catch (Figure 5.11 A, B). Pair-wise comparison by block of treated and control traps for each trap type showed that the aggregation pheromone, neryl (*S*)-2-methylbutanoate, increased trap catch of females by $\times 3.1$, and of males by $\times 2.5$ (Mann Whitney, $W_{20} = 302$, $P = 0.003$ for females; $W_{20} = 317$, $P = 0.009$ for males) in the delta traps, but the increase in trap catch on the sticky traps, by $\times 1.3$ (females) and $\times 1.1$ (males), were not statistically significant (Mann Whitney, $W_{20} = 353$, $P = 0.13$ for females; $W_{20} = 402$, $P = 0.83$ for males). A comparison of the ratio of treatment: control catch of adult thrips by block for the different trap types showed a significant interaction between the effect of the pheromone and the attractiveness of the trap (Mann-Whitney, $W_{20} = 308$, $P = 0.006$). The pheromone increased trap catch in inverse proportion to the attractiveness of the trap, with a median ratio (treatment: control) of $\times 2.2$ in delta traps and of $\times 1.3$ on sticky traps. Although the brown delta trap enhanced the response to pheromone, further experiments are required to determine whether this was the result of trap type or trap colour.

5.3.6. Does trap height above the crop affect pheromone trap catch?

There was a significant difference in trap catch between treatments overall for females (ANOVA, ($F_{(1, 57)} = 30.1$, $P < 0.001$) and males (ANOVA, $F_{(1, 57)} = 68.3$, $P < 0.001$). Traps that were closer to the crop caught $\times 1.7$ more females and $\times 2.2$ more males than traps that were higher above the crop (Figure 5.12 A, B). The aggregation pheromone, neryl (*S*)-2-methylbutanoate, increased trap catch of females by $\times 1.4$ (ANOVA, $F_{(1, 57)} = 13.4$, $P < 0.001$), and of males by $\times 1.2$ (ANOVA, $F_{(1, 57)} = 4.9$, $P = 0.032$). There was no significant interaction between response to pheromone at the different trap heights (females, ANOVA, $F_{(1, 57)} = 0.4$, $P = 0.55$; males, ANOVA, $F_{(1, 57)} = 0.1$, $P = 0.72$) indicating that neither the trap colour nor the pheromone are drawing the thrips away strongly from the crop canopy.

5.3.7. Does trap spacing affect pheromone trap catch?

There was a significant difference in trap catch between treatments overall for females (ANOVA, $F_{(3, 57)} = 14.6$, $P < 0.001$) and males (ANOVA, $F_{(3, 57)} = 24.4$, $P < 0.001$). Overall, the aggregation pheromone, neryl (*S*)-2-methylbutanoate, increased trap catch of females by $\times 1.4$ and of males by $\times 1.2$. There was a trend for a greater treatment: control ratio with increasing distance between traps ($\times 1.2$ near and $\times 1.5$ far) (Figure 5.13 A, B). In pair-wise Tukey's comparisons there was no statistical difference in the trap catch between treatment and control traps placed 1.2 m apart (females, $P = 0.68$; males $P = 0.64$), whereas the difference was significant at 6 m spacing (females, $P < 0.001$; males $P = 0.011$), showing that the pheromone influenced the control trap catch when the traps were 1.2 m apart but not (or to a lesser extent) when they were 6 m apart. The interaction between spacing and pheromone was significant for females ($F_{(1, 57)} = 4.1$, $P = 0.049$) but not males ($F_{(1, 57)} = 0.4$, $P = 0.52$). The control traps that were furthest from a pheromone lure (6 m) caught the fewest thrips, as predicted.

5.3.8. Is response to pheromone synergised by a plant volatile analogue?

5.3.8.1. In a pepper crop in Spain

Small increases in trap catch were observed with the different scents (Figure 5.14 A, B): The increase in trap catch with methyl isonicotinate ($\times 1.2$, females and males combined) was not statistically significant; neryl (*S*)-2-methylbutanoate increased trap catch significantly by $\times 1.3$ (females and males combined); the combination of pheromone and methyl isonicotinate increased trap catch by $\times 1.4$ (females and males combined), but there was no synergistic effect over and above the multiplicative effects of the combination. There was a significant difference in trap catch between treatments overall for females (ANOVA, $F_{(3, 57)} = 6.4$, $P = 0.001$), but not males (ANOVA, $F_{(3, 57)} = 2.0$, $P = 0.12$), although the trends were similar in both sexes. Analysis of treatment effects of the two compounds was repeated using two-factor ANOVA with contrasts, to take account of the scents in the mixed treatment, which showed no significant increase in trap catch with methyl isonicotinate (females, $F_{(1, 57)} = 0.9$, $P = 0.36$; males, $F_{(1, 57)} = 0.1$, $P = 0.76$), a significant increase in trap catch with neryl (*S*)-2-methylbutanoate (females, $F_{(1, 57)} = 18.1$, $P < 0.001$; males, $F_{(1, 51)} = 5.8$, $P = 0.02$) and no interaction between the two scents (females, $F_{(1, 57)} = 0.2$, $P = 0.64$; males, $F_{(1, 51)} = 0.1$, $P = 0.71$).

5.3.8.2. In a strawberry crop in the UK

When the experiment was repeated in UK strawberry, both scents significantly increased trap catch by similar amounts (Figure 5.15 A, B): Methyl isonicotinate increased the trap catch of females by $\times 1.4$ and of males by $\times 1.3$; neryl (*S*)-2-methylbutanoate increased trap catch of females by $\times 1.3$ and of males by $\times 1.2$; the combination of pheromone and methyl isonicotinate increased trap catch by $\times 1.4$ (females and males combined), but there was no synergistic increase in trap catch over and above the multiplicative effects of the combination. There was a significant difference in trap catch between treatments overall for females (ANOVA, $F_{(3, 51)} = 6.4$, $P < 0.001$), and males (ANOVA, $F_{(3, 51)} = 10.8$, $P < 0.001$). Analysis of treatment effects of the two compounds was repeated using two-factor ANOVA with contrasts, to take account of the scents in the mixed treatment, which showed a significant increase in trap catch with methyl isonicotinate (females, ANOVA, $F_{(1, 51)} = 40.3$, $P < 0.001$; males, ANOVA, $F_{(1, 51)} = 25.6$, $P < 0.001$), a significant increase in trap catch with neryl (*S*)-2-methylbutanoate (females, ANOVA, $F_{(1, 51)} = 9.2$, $P = 0.004$; males, ANOVA, $F_{(1, 51)} = 4.1$, $P = 0.049$). There was significant interaction between the two scents for females (ANOVA, $F_{(1, 51)} = 9.5$, $P = 0.003$), which is a synergistic decrease, because the combined effect of the two scents was significantly less than the product of their separate effects on log-transformed data. No significant interaction was observed in males (ANOVA, $F_{(1, 51)} = 2.7$, $P = 0.10$).

5.3.9. Thrips species composition

The Thripidae species composition was similar between the two experimental sites in Spain, with about 97 % *F. occidentalis* and 2% *T. tabaci*. *Thrips angusticeps* and *F. intonsa* were also present in low numbers (<1%). Occasional aeolothripid and phlaeothripid thrips were present on most traps, including the predatory species *Aeolothrips tenuicornis*.

In the UK strawberry crop, (see 5.3.8.2), 97% of the thripids in flowers were *F. occidentalis* (n = 100) and 100% of the thripids identified from traps were *F. occidentalis* (n = 18), with an average of 16 adult thrips per flower and 1 flower per plant (n = 10 flowers and plants).

5.4. Discussion

The results confirm that the aggregation pheromone, neryl (*S*)-2-methylbutanoate, increases trap catch of both male and female *F. occidentalis* in protected pepper crops in Spain and a semi-protected strawberry crop in the UK. The increase in trap catch is consistent with that found previously in greenhouse pepper, tomato, cucumber and outdoor top fruit, where addition of the pheromone resulted in increases between $\times 1.2$ and $\times 3$ (Hamilton *et al.*, 2005; Gómez *et al.*, 2006; Broughton & Harrison, 2012; Covaci *et al.*, 2012). The experiments in strawberry in this thesis and resultant publications are the first records of increased trap catch using the aggregation pheromone in semi-protected strawberry, showing that the pheromone could be used to enhance trapping and monitoring in strawberry.

The increase in trap catch with the aggregation pheromone was consistently greater for females ($\times 1.7$) than for males ($\times 1.4$) (mean of nine experiments). The greater increase in trap catch of females in the field could reflect a higher trap catch of virgin females, although this is untested. Alternatively, as most females are likely to be mated (Terry & Schneider, 1993), the greater increase in trap catch for females with the pheromone could be because females disperse more readily than males (Terry & Gardner, 1990; Rhainds & Shipp, 2003). Males have a more aggregated distribution than females, as shown by the coefficients of variation (standard deviation/mean), which were higher (more variable) in males than in females in all experiments. To give a typical example (experiment 5.3.3.1), the coefficient of variation (CV) was 73% for males and 65% for females on control traps, and 64% for males and 48% for females on pheromone traps. The reduction in the CV with the aggregation pheromone is unexplained. It might be that the control traps show the underlying distribution of thrips within a crop while pheromone traps reflect overall activity over a crop and that the two types of trap are not sampling the same sets of the population. It is not clear why so many non-virgin females are attracted to the aggregation pheromone given that they do not need to mate to lay eggs, that most are likely to have already mated (Terry, 1997), and that they do not mate again for several days after mating (Terry & Schneider, 1993).

Frankliniella occidentalis trap catch was not affected by dose of neryl (*S*)-2-methylbutanoate over the wide range tested (10-2000 μg). This may be expected theoretically because the pheromone is produced in variable amounts, because thrips

numbers vary within aggregations and there is no evidence of a negative feedback mechanism for pheromone production rate. The response to pheromone could vary at doses above or below those tested. The lowest dose tested (10 µg) had the lowest trap catch, so there is nothing to suggest a lower dose would increase trap catch (Figure 5.4). Its release rate of about 0.04 µg per day (D. Hall, pers. comm., 2011) is equivalent to the amount of pheromone produced by about 6-17 male thrips per day, calculated up from a release rate of 100-300 pg per male per h (Dublon *et al.*, 2008). This is only an approximate measure for the purposes of comparison, because pheromone production rate is variable and as it is not known how the thrips pheromone production varies through the day. De Kogel (2003) found a positive dose response of females to increasing numbers of male *F. occidentalis* from 5-80 males, although other compounds may be involved when using live thrips. There is no reason to suppose that doses higher than those tested would increase trap catch as there was no trend towards increased trap catch with doses between 100 and 2000 µg (Figure 5.4). As no critical dose was identified it suggests that thrips are not responding to aggregation pheromone in the same way that Lepidoptera respond to their sex pheromones. Lepidoptera use concentration to locate conspecific mates and land at specific concentrations (Cardé & Baker, 1984), whereas thrips land at various concentrations. As most moth species are nocturnal, scents are likely to be more important for mate location than for day-flying thrips, which use visual cues for landing. There is an apparent advantage for thrips in responding to high doses of pheromone because they have more chance of mating in aggregations that contain more thrips (Milne *et al.*, 2002). A possible benefit of the lack of a dose-response in thrips is that the dose rate for lures is less critical, so there is more margin for error than in moth species that land in response to specific doses. As there is no evidence that trap catch would increase by altering the dose, the dose used in commercial lures of 30 µg per septa (equivalent to 100 µg in vials) is adequate.

The chiral form of neryl 2-methylbutanoate was critical to the response as is typical of insect pheromones and the experiments confirm neryl (*S*)-2-methylbutanoate as the aggregation pheromone, which increases trap catch. The experiments also demonstrate that neryl (*R*)-2-methylbutanoate is biologically active, reducing or cancelling out the effect of neryl (*S*)-2-methylbutanoate at very low concentration. It is not known why neryl (*R*)-2-methylbutanoate is affecting trap catch. It could be working by interfering with the receptors to neryl (*S*)-2-methylbutanoate, or could play an active role as a repellent. There

is no evidence that the (*R*) form is produced by *F. occidentalis* or *F. intonsa* (Hamilton *et al.*, 2005; Zhu *et al.*, 2012), although it could be produced by related species and have a role that has not been identified. The small amounts of neryl (*R*)-2-methylbutanoate (about 1%) present in commercial sources of neryl (*S*)-2-methylbutanoate are unlikely to have a great impact on trap catch, although the purity of the pheromone source will affect efficacy. Olaniran (2013) reported that the response of female *F. occidentalis* (walking and flitting) to filter paper dosed with synthetic pheromone failed to match that to filter papers that had been exposed to live thrips in laboratory experiments, because 7-methyltricosane was absent. Contamination of commercial sources of neryl (*S*)-2-methylbutanoate with small amounts of the (*R*)-form may contribute as it would reduce the response to the aggregation pheromone, although other compounds and behaviours may be involved.

(*R*)-lavandulyl acetate did not increase trap catch at any of the ratios (to the aggregation pheromone) or doses tested, so there is no evidence for the suggestion by Zhu *et al.* (2012) that it is part of the aggregation pheromone. Zhu *et al.* (2012) suggested that the ratio and doses of the two compounds play an important role in interspecies recognition between *F. occidentalis* and *F. intonsa*, as *F. intonsa* produce more of both compounds and proportionally more (*R*)-lavandulyl acetate than *F. occidentalis*. As the ratios between the two compounds and release rates tested extended over the full range entrained from males of both species, it seems unlikely that a critical ratio or dose has been missed. The significant reduction in trap catch with (*R*)-lavandulyl acetate in females (Figure 5.9 A) indicates that it has a role of its own, rather than reducing trap catch by direct interference with the aggregation pheromone. In contrast its enantiomer, (*S*)-lavandulyl acetate, did not affect the trap catch (no increase or decrease) of females or males, suggesting that it is not biologically active. Olaniran (2013) found that (*R*)-lavandulyl acetate arrested females, reducing walking and flits in laboratory experiments and suggested that it may be used by males to calm females during mating. Pelikan (1951) observed that adult male *Pezothrips dianthi* (formerly *Taeniothrips dianthi*) also produce a compound that calms females. The reduction in female *F. occidentalis* trap catch in response to (*R*)-lavandulyl acetate might, therefore, be as a result of females landing (arresting) before they arrive at a trap, or failing to take off near the trap. Similar compounds have been identified in some mealybug and scale insect species that reduce trap catch and play a role in copulation behaviour (Millar *et al.*, 2005). In most experiments the effect of (*R*)-lavandulyl acetate on male trap catch was

not statistically significant (Figure 5.9 B). Olaniran (2013) found increased activity of males (walking and flits) in response to the compound in laboratory experiments, which he associated with fighting and mating behaviour, but this increased activity did not translate into increased trap catch in the field, rather there was a slight decrease in trap catch with a similar trend to that in females, which is unexplained. Further work is required to identify the biological role of (*R*)-lavandulyl acetate. A bioassay to test whether mating success is improved in the presence of the compound should be sufficient to confirm whether it aids mating as suggested by Olaniran (2013). As it does not appear to be part of the aggregation pheromone, there is no benefit in adding (*R*)-lavandulyl acetate to pheromone lures to increase trap catch of *F. occidentalis*. Further experiments are required to test whether the response of females to (*R*)-lavandulyl acetate is sufficient to reduce feeding damage and egg-laying. This could be a new method of pest control with an applied use which is worth investigating.

The results on different trap colours confirmed the attractiveness of blue (about 450 nm, see Chapter 6) to *F. occidentalis* in greenhouse crops (Brødsgaard, 1989; Vernon & Gillespie, 1990) and the importance of visual cues in trap attraction. The absolute increase in trap catch with additional pheromone was greater for the more attractively coloured traps, although the proportional increase was lower. A similar interaction between colour and scent has been observed with plant kairomones (Teulon *et al.*, 1999; Davidson *et al.*, 2012). The reduced treatment: control ratio with pheromone on the most visually attractive traps may be explained, at least in part, by the fact that they are already extremely attractive, drawing in a large number of thrips, thereby reducing the numbers of thrips available for the pheromone to attract unless an invasion of thrips is occurring. For example, the blue traps were catching thrips equivalent to about all the adult thrips in flowers of about 5 m² of crop (19 thrips per m² in flowers (site 1, Table 5.1); 99 thrips per blue trap (Figure 5.10)). The black pheromone traps and brown delta traps caught very few thrips, confirming that a visual cue is an essential part of the traps. The pheromone is known to attract *F. occidentalis* in olfactometer experiments (although the thrips are mostly walking rather than flying) (Hamilton *et al.*, 2005) and it increases activity (including flits) (Olaniran, 2013), so it is possible that thrips take off and fly towards the pheromone lures, but landed on attractively coloured surfaces (such as the white flowers in the pepper crop) before reaching the less attractively coloured (black or brown) traps. No conclusions can be made about whether the delta trap design increases pheromone trap

catch as there was no comparison of sticky traps and delta traps of the same colour, with and without pheromone. The treatment: control ratio was about the same (3:1 for adult thrips) in the brown delta traps and on the black traps (i.e. less attractive colours) in different experiments, indicating that the low trap catches and higher treatment: control ratio may be the result of trap colour rather than trap type. Further experiments (for example with blue delta traps) are required to test this, although, as the trap catch was so low in the delta traps (35% of the control traps caught no thrips) and as no great increase was seen with added pheromone, they are unlikely to be a useful tool for thrips monitoring compared to sticky traps, which are cheaper. Blue sticky traps (about 450 nm) with added pheromone resulted in the highest trap catches of the different colours tested and these were used for mass trapping (see Chapter 6).

The plant volatile analogue, methyl isonicotinate and the aggregation pheromone increased the trap catch of *F. occidentalis* by similar amounts in UK strawberry, which is consistent with results in top fruit in Australia (Broughton & Harrison, 2012). Both scents increase thrips activity and take-off (van Tol *et al.*, 2012; Olaniran, 2013). The lack of a synergistic increase in trap-catch with the two scents combined is similar to the response observed in *T. tabaci* when different plant volatiles were combined (Teulon *et al.*, 2007c). The lack of response by a Spanish thrips population to methyl isonicotinate in a pepper crop is consistent with that found by Nielsen (pers. comm., 2013). There is the possibility that thrips could habituate to certain scents or that population control using scents could lead to adaptive changes (resistance). This could apply equally to plant volatiles and pheromones, as the pheromone composition within a species can change between populations, for example, the ratio of different pheromone components in the turnip moth (*Agrotis segetum*) varies between European countries (Löfstedt *et al.*, 1986). If changes in the pheromone composition occur in response to trapping, then the techniques need to change along with the insect development. Resistance management may need to be considered in the same way as insecticides, considering the frequency of application or rotation between scents. An alternative explanation for a lack of response to different scents could relate to the cropping environment, as fewer thrips respond to traps in more attractive environments (Prokopy & Owens, 1983). For example, the plant volatile *p*-anisaldehyde did not increase trap catch in UK strawberry (Sampson, unpublished data, 2013), possibly because *p*-anisaldehyde is already present in strawberry flowers and one of the behavioural responses to *p*-anisaldehyde is reduced take-off (arrestment) (Teulon *et al.*,

1999; Davidson *et al.*, 2012). Further controlled experiments are required to understand the flight response of *F. occidentalis* to lures and competing scents (from the crop, weeds and other thrips) at different times of the year in the field, so that the use of scents can be optimised for trapping.

The experiments in this study fail to identify any improvements to the aggregation pheromone for trapping, but they do provide further clues as to how the thrips are responding to the pheromone. The significant effect of trap spacing on trap catch (Figure 5.13), where the pheromone affected trap catch on control traps that were 1.2 m away but not (or to a lesser extent) at 6 m away, is proof that the thrips are landing in response to colour rather than flying directly to the scent, because the scented trap has increased the trap catch of an unscented trap that is 1.2 m away. The landing of thrips in response to visual stimuli could partly explain why the increase in thrips trap catch is not higher in flowering pepper and strawberry crops in this study, because some thrips may land on the white flowers that were abundant throughout the crop (a mean of 5 and 10 flowers per m² in the pepper and strawberry crops respectively at the time of the experiments), before reaching a trap. In contrast, some nocturnal lepidopteran species are more attracted to scent than to visual cues, landing at exact concentrations of pheromone and continuing to search until the source of a scent is found, which results in a greater increase in trap catch compared to controls (Howse, 1998). The pheromone treatment effect in this study was no different when traps of the same colour were 6 m or 3.6 m apart in different experiments (e.g. about $\times 1.3$ increase in blue trap catch of adults thrips with pheromone in 5.3.4 and 5.3.7), suggesting that there was minimal spread of pheromone scent onto the control traps at these distances. This would indicate that minimum pheromone lure spacing should be somewhere between 2.4 and 6 m for maximum effect, based on evidence that pheromone boosted control traps that were 1.2 m away, but had a limited effect of pheromone on control traps that were 6 m away. Teulon *et al* (2007a) predicted a similar effect of ethyl isonicotinate on *T. tabaci*, where trap catch was predicted to decrease by 50% within 1.3 m of a baited trap. Further studies are required to identify the best spacing for lures for monitoring and mass trapping, as the distance over which thrips respond to scents in the field is unknown.

It is evident that not all thrips are responding to the pheromone traps as there were plenty of thrips in the flowers immediately surrounding a trap in the field. The thrips caught on a trap may include a few more responsive thrips that are flying in from further

distances as well as those immediately surrounding a trap. The most effective hue (dominant wavelength) is very attractive to *F. occidentalis* (Brødsgaard, 1989), yet neither an attractive hue nor pheromone drew thrips away from the flowering canopy when traps were raised higher above the crop (Figure 5.12). Thrips may be arrested in the flowers, rather than flying towards the traps, as flowers are attractively coloured and may contain floral scents, pheromone producing thrips, and a favourite food source (pollen). Both *p*-anisaldehyde (which is present in strawberry flowers) and (*R*)-lavandulyl acetate (produced by male *F. occidentalis*) are known to arrest thrips (Teulon *et al.*, 1999; Olaniran, 2013), which could explain the lack of response in some thrips, although various other abiotic (e.g. temperature and wind speed) and biotic (e.g. crowding and hunger status) factors are likely to be involved. As no dose-related response to pheromone was identified in thrips, the higher dose from a pheromone trap may not override the attraction of other thrips within a flower, or it may be that thrips only respond when in flight. The release rate from commercial lures (about 0.4 µg per day, D. Hall, pers. comm., 2011) is equivalent to that produced by about 60-170 male thrips per day, which sounds a lot, but adult trap catches in UK strawberry can exceed 2000 thrips per week during the summer (see Chapter 3), so there is considerable competition from pheromone produced by thrips within the crop. A greater pheromone trap catch compared to control would be expected in a non-flowering crop because of reduced competition from the environment and because the thrips are likely to be less satiated and therefore flying more (Davidson *et al.*, 2006, 2009) and this is explored further in Chapter 6. The proportion of immigrant thrips was unknown in the crops in this study, but a greater response to trapping might occur when there are more immigrants. Further knowledge about the movement of thrips within and between crops would help to identify the best timing and placement of traps.

The *F. occidentalis* aggregation pheromone, neryl (*S*)-2-methylbutanoate, resulted in a small, but consistent increase in trap catch throughout the study, with a greater increase of females than males. (*R*)-lavandulyl acetate did not increase trap catch at any of the ratios (to the aggregation pheromone) or doses tested, so there is no evidence that it is part of the aggregation pheromone. No other volatile components of sufficient volume to attract thrips over a distance have been found from the head space of *F. occidentalis* (Hamilton *et al.*, 2005; Olaniran, 2013), so although it is possible that other compounds may be involved in aggregation, it seems unlikely. Further testing of the less volatile cuticular hydrocarbons, which act on contact or at short distances, is required to determine whether

they could be used to improve trap catch. Applied use of the pheromone has yet to be fully explored. Even a small increase in trap catch can be important to improve the sensitivity and reliability of monitoring traps, especially early in the season, when it is important to apply timely releases of natural enemies. There is also potential for mass trapping (see Chapter 6), attract and kill, attract and infect (Niassy *et al.*, 2012) or to improve the efficacy of insecticide treatments in a similar way to alarm pheromones (Cook *et al.*, 2002).

Table 5.1. Field site details showing location, growing methods, environmental conditions and thrips occupation, for the thrips trapping experiments carried out in protected sweet pepper crops in Spain, April 2011.

| | Site 1 | Site 2 |
|-----------------------------|-------------------------------|-------------------------------|
| Location | El Algar, Murcia, Spain | Los Infiernos, Murcia, Spain |
| Grid Reference | N 37° 37.34' W 0° 53.48' | N 37° 57.07' W 0° 54.77' |
| Structure | Multi-tunnel plastic house | Multi-tunnel plastic house |
| Area | 7500 m ² | 6000 m ² |
| Cultivar | Velez | Guepard |
| Growing method | Hydroponic | In soil |
| Planting density | 2.5 plants per m ² | 2.5 plants per m ² |
| Crop height | 66 cm | 90 cm |
| Mean min. and max. temp. | 14-37 °C | 14-36 °C |
| Mean min. and max. RH | 34-89 % | 36-88 % |
| Thrips occupancy of flowers | 40% | 35% |
| Thrips per flower | 3.1 | 1.2 |
| Flowers per plant | 2.5 | 6 |

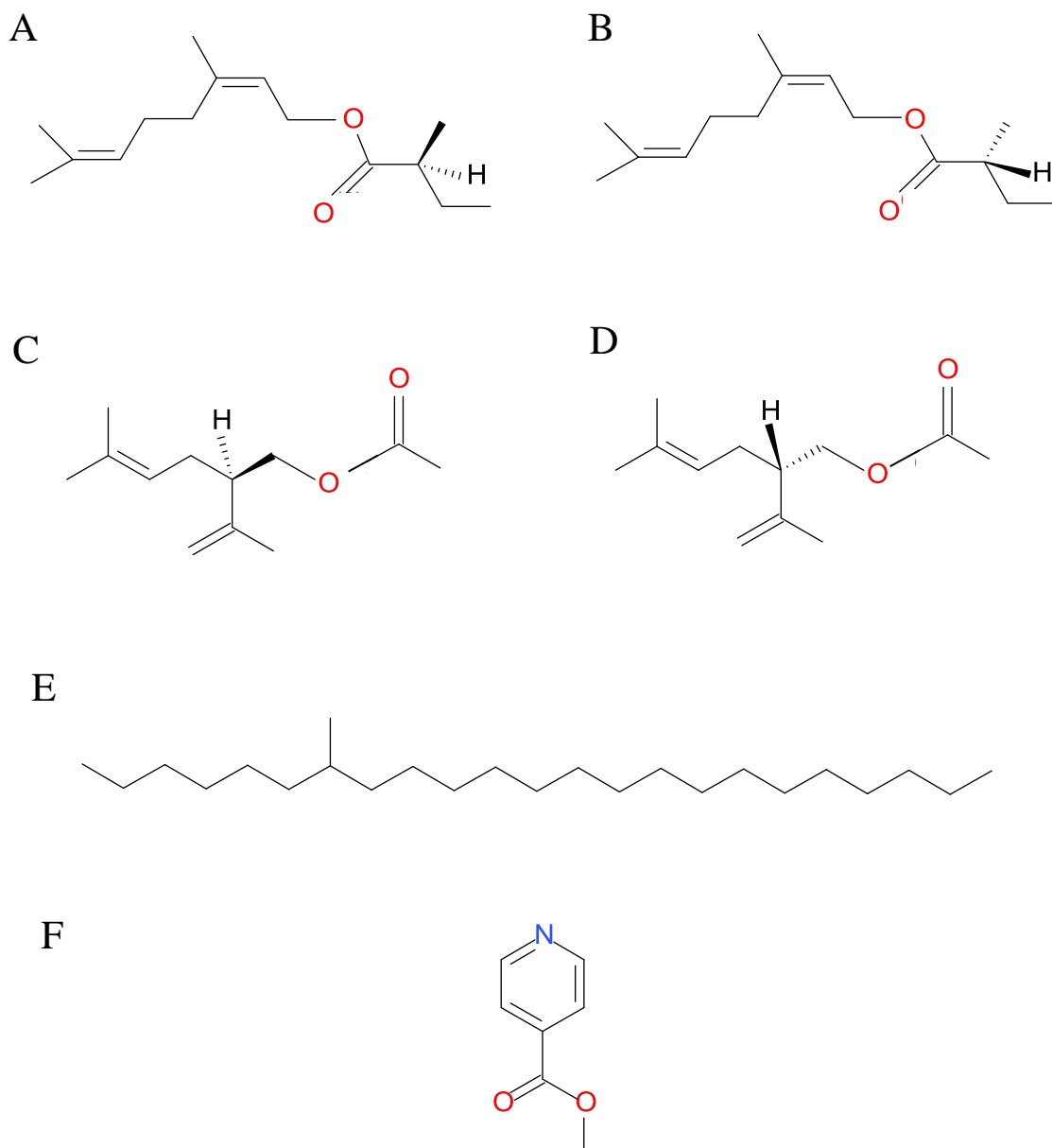


Figure 5.1. Chemical structure of : (A) neryl (*S*)-2-methylbutanoate; (B) neryl (*R*)-2-methylbutanoate; (C) (*R*)-lavandulyl acetate; (D) (*S*)-lavandulyl acetate; (E) 7-methyltricosane; (F) methyl isonicotinate.

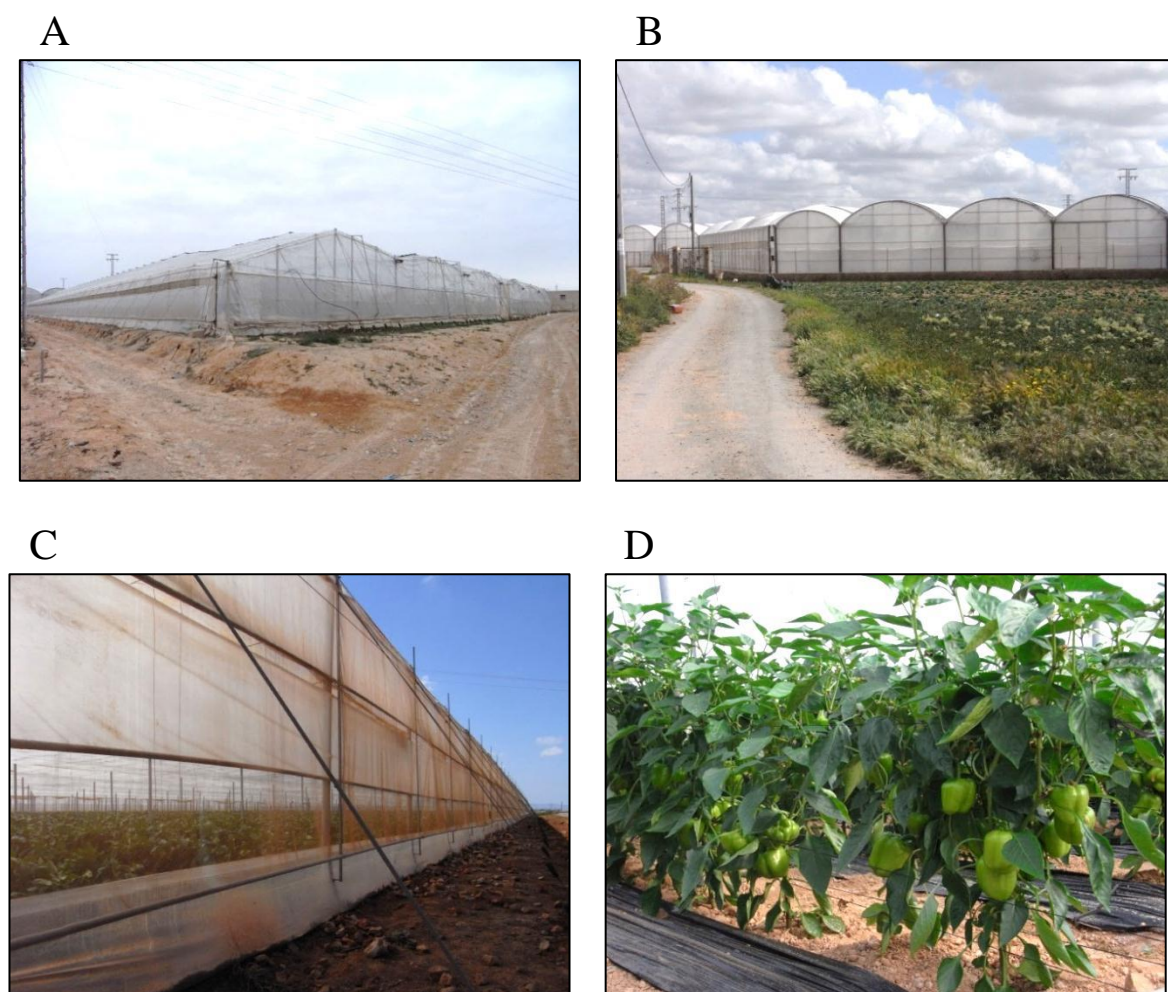


Figure 5.2. Field sites for the pheromone trap trials showing (A) site 1 (B) site 2 (C) vents at site 1 (D) pepper plants growing at site 2.

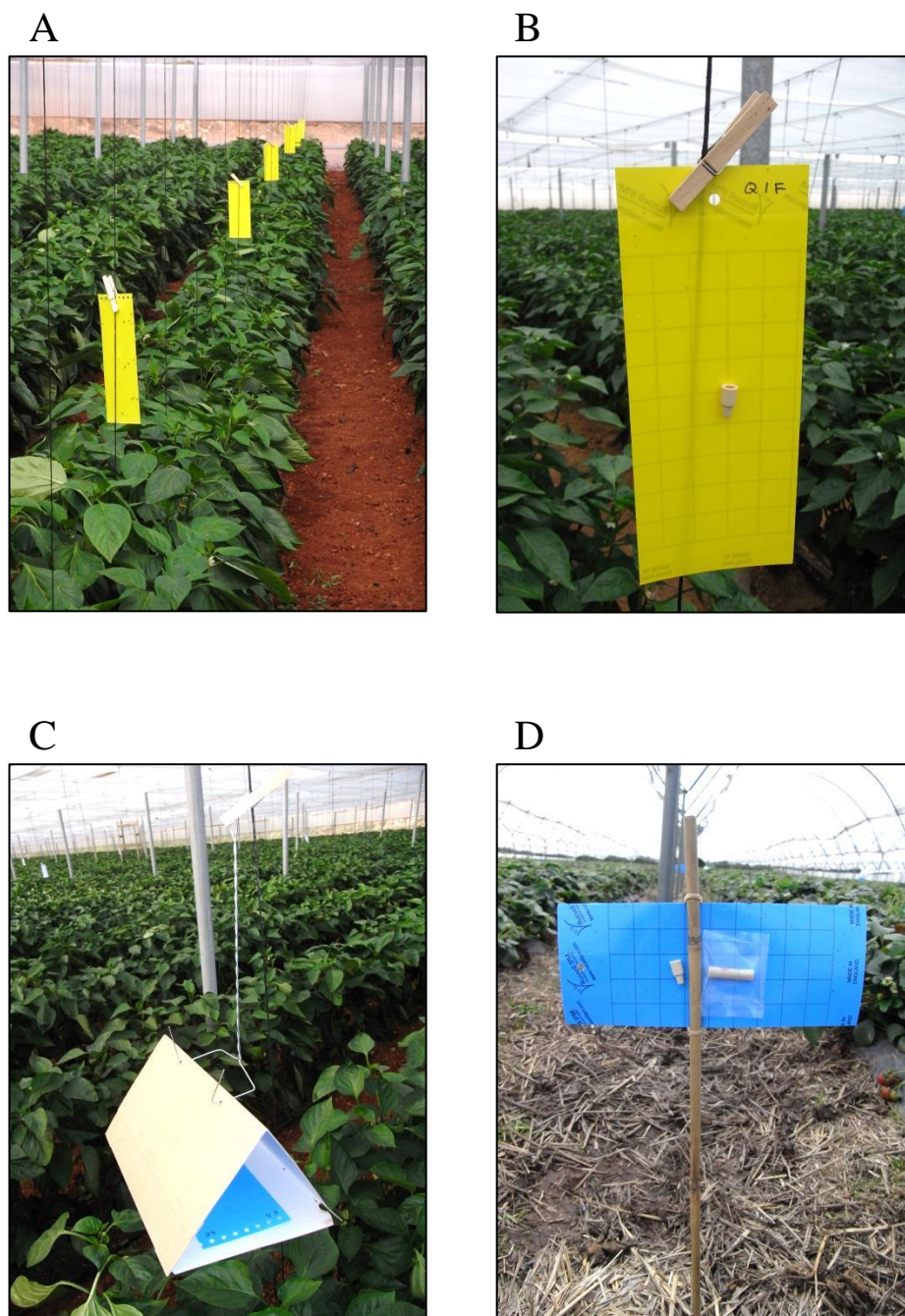


Figure 5.3. Field experiments showing (A) a typical block lay-out in a Spanish pepper pheromone experiment (B) the position of the lures on the traps (C) a delta trap with a blue sticky trap insert and (D) a blue sticky trap with a septum and sachet in the UK strawberry experiment.

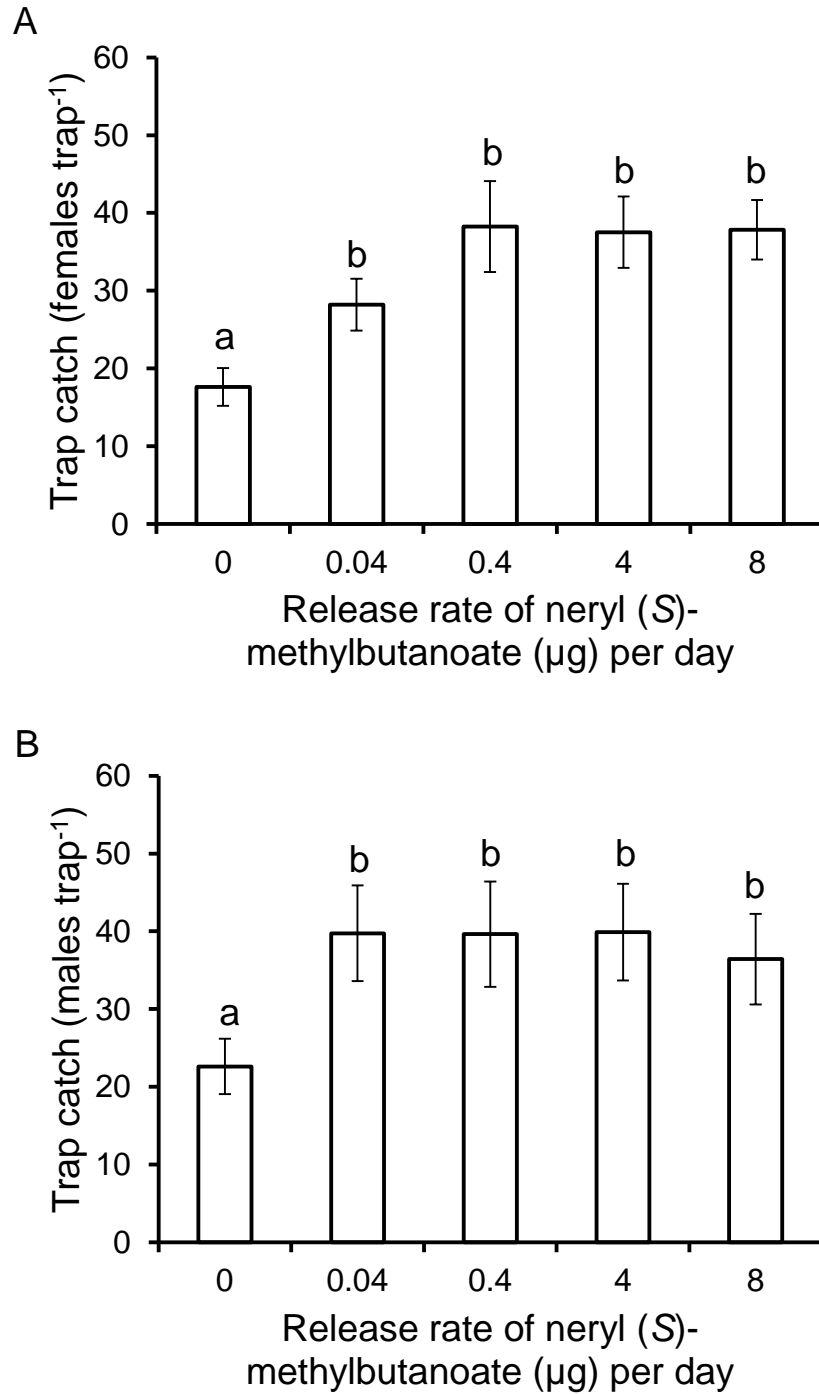


Figure 5.4. The mean number \pm SEM of adult *F. occidentalis* caught on yellow sticky traps with different release rates of neryl (*S*)-2-methylbutanoate from vials, over 24 h in a pepper crop ($n = 19$) for (A) females ($F_{(4, 72)} = 13.4$, $P < 0.001$) and (B) males ($F_{(4, 72)} = 4.9$, $P = 0.002$). The release rates equated to doses of 0, 10, 100, 1000 and 2000 µg per vial. Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

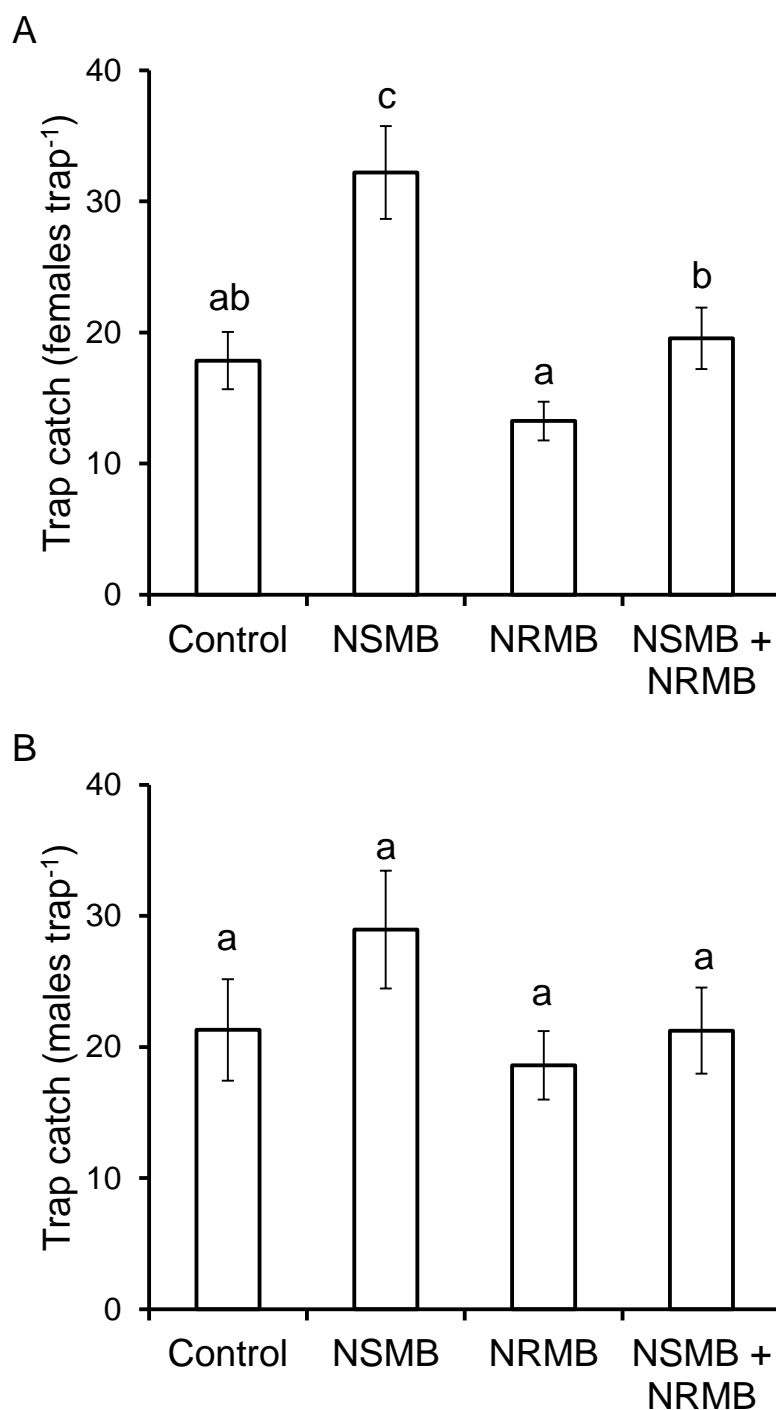


Figure 5.5. The mean number \pm SEM of adult *F. occidentalis* caught on yellow sticky traps with different chiral forms of neryl 2-methylbutanoate, using vials, over 24 h in a pepper crop ($n = 20$) for (A) females ($F_{(3,57)} = 6.7$, $P < 0.001$) and (B) males ($F_{(3,57)} = 2.6$, $P = 0.065$). NSMB = neryl (*S*)-2-methylbutanoate, NRMB = neryl (*R*)-2-methylbutanoate. The release rate of the compounds was about 0.4 μg per day from vials. Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

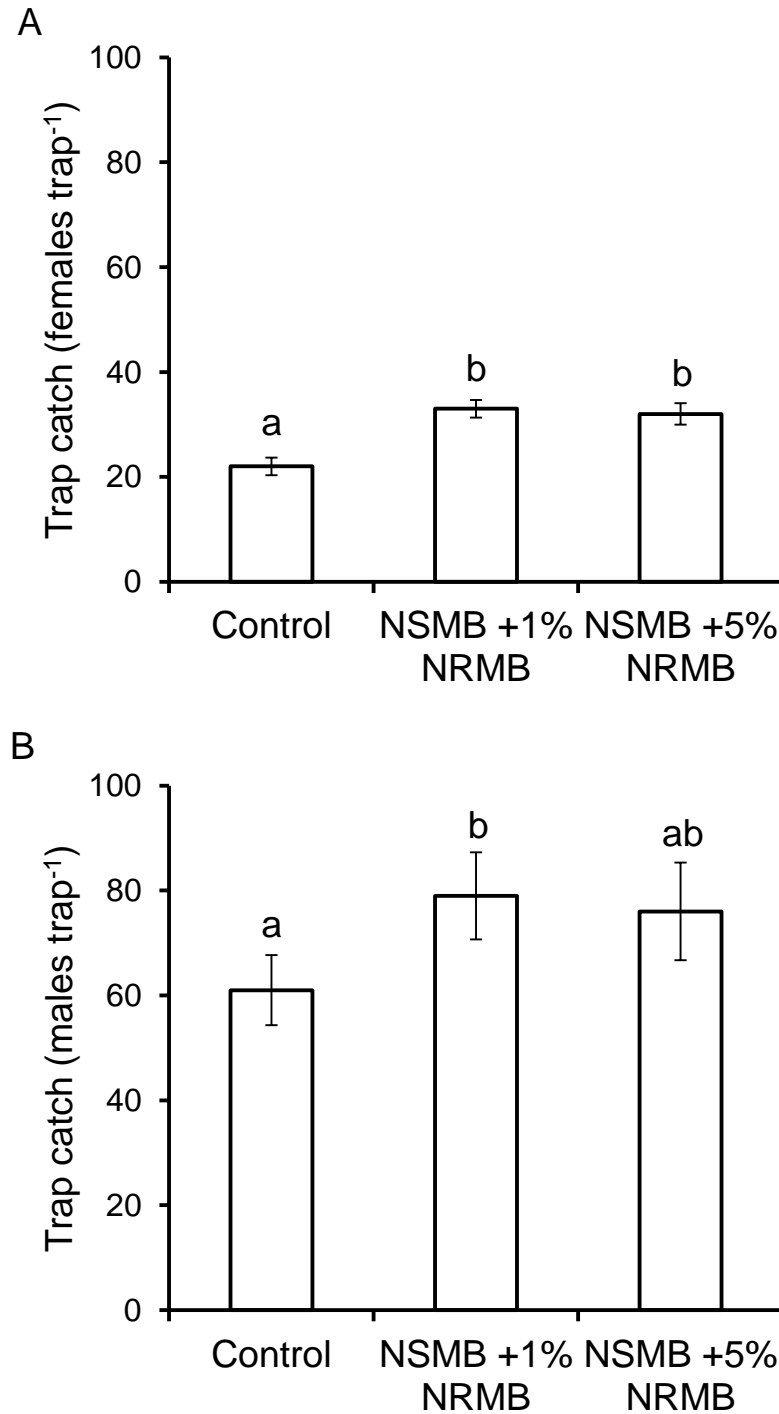


Figure 5.6. The mean number \pm SEM of adult *F. occidentalis* caught on yellow sticky traps with an additional small amount (4%) of neryl (*R*)-2-methylbutanoate added to the 1% already present in the (*S*) form, using septa, over 24 h in a pepper crop ($n = 20$) for (A) females ($F_{(2, 38)} = 19.0$, $P < 0.001$) and (B) males ($F_{(2, 38)} = 4.1$, $P = 0.026$). NSMB = neryl (*S*)-2-methylbutanoate, NRMB = neryl (*R*)-2-methylbutanoate. Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

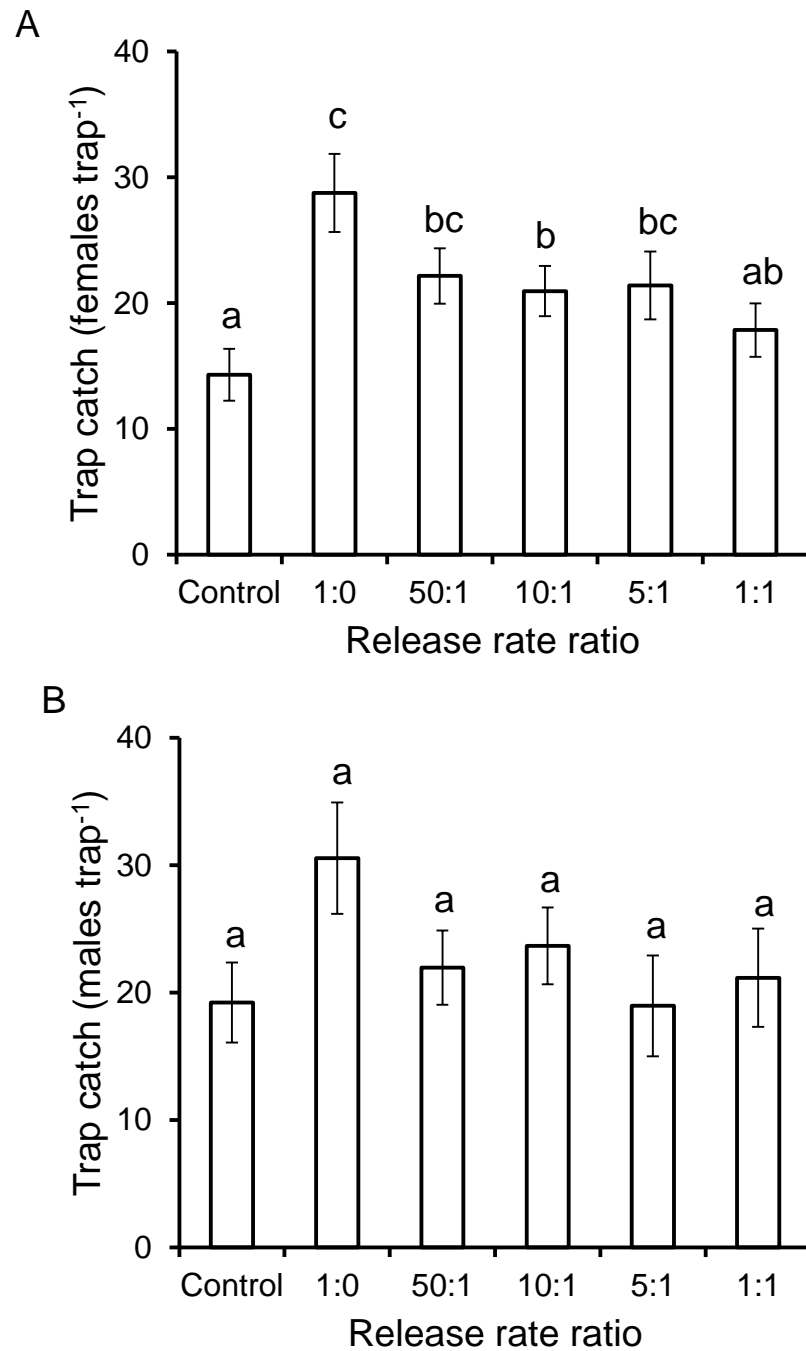


Figure 5.7. The mean number \pm SEM of adult *F. occidentalis* caught on yellow sticky traps with different release rate ratios of neryl (S)-2-methylbutanoate: (R)-lavandulyl acetate from vials, over 24 h in a pepper crop ($n = 20$) for (A) females ($F_{(5, 95)} = 11.0$, $P < 0.001$) and (B) males ($F_{(5, 95)} = 1.6$, $P = 0.17$). The release rate of neryl (S)-2-methylbutanoate was about $0.4 \mu\text{g}$ per day for all treatments (except the control), with proportional release rate of (R)-lavandulyl acetate. Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

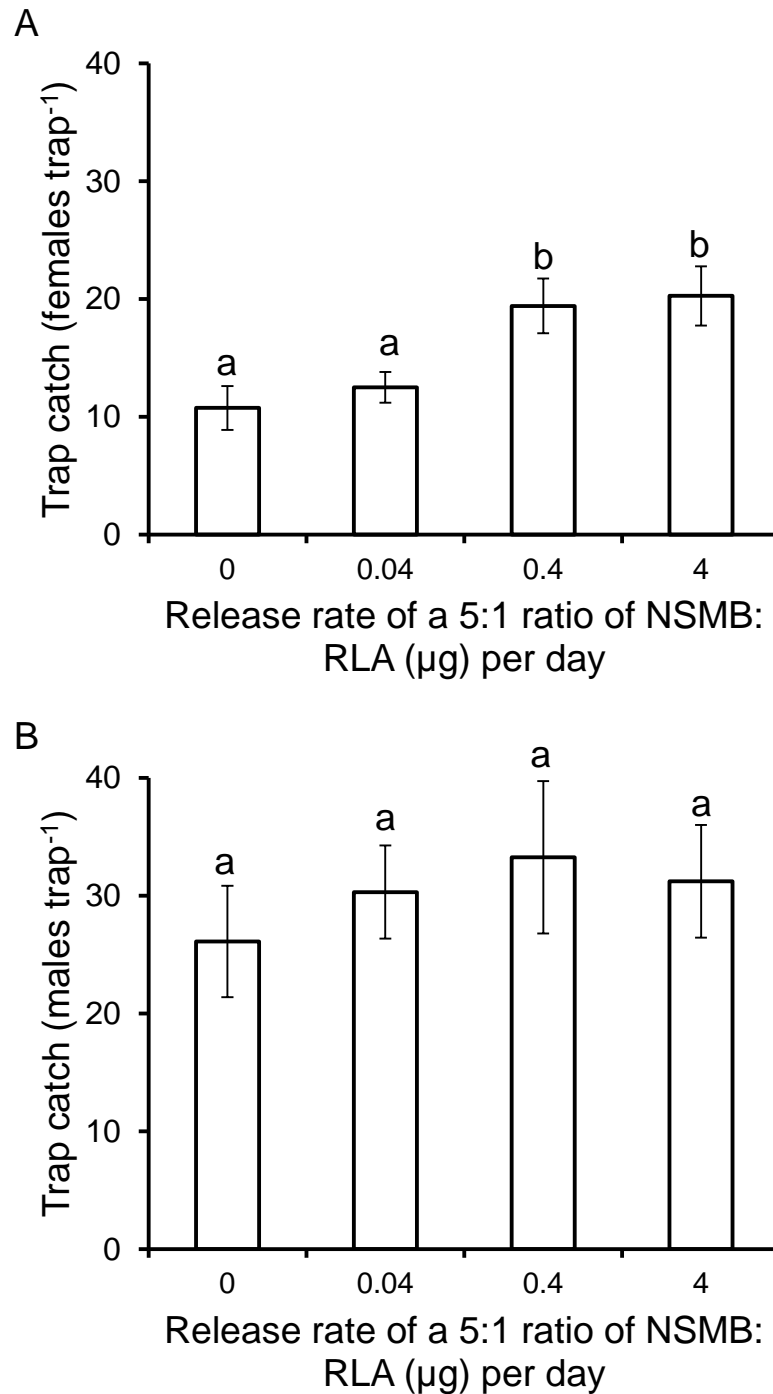


Figure 5.8. The mean number \pm SEM of adult *F. occidentalis* caught on yellow sticky traps with different release rates of a 5:1 ratio of neryl (S)-2-methylbutanoate: (R)-lavandulyl acetate from vials, over 24 h in a pepper crop ($n = 20$) for (A) females ($F_{(3, 57)} = 21.6$, $P < 0.001$) and (B) males ($F_{(3, 57)} = 1.1$, $P = 0.38$). Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

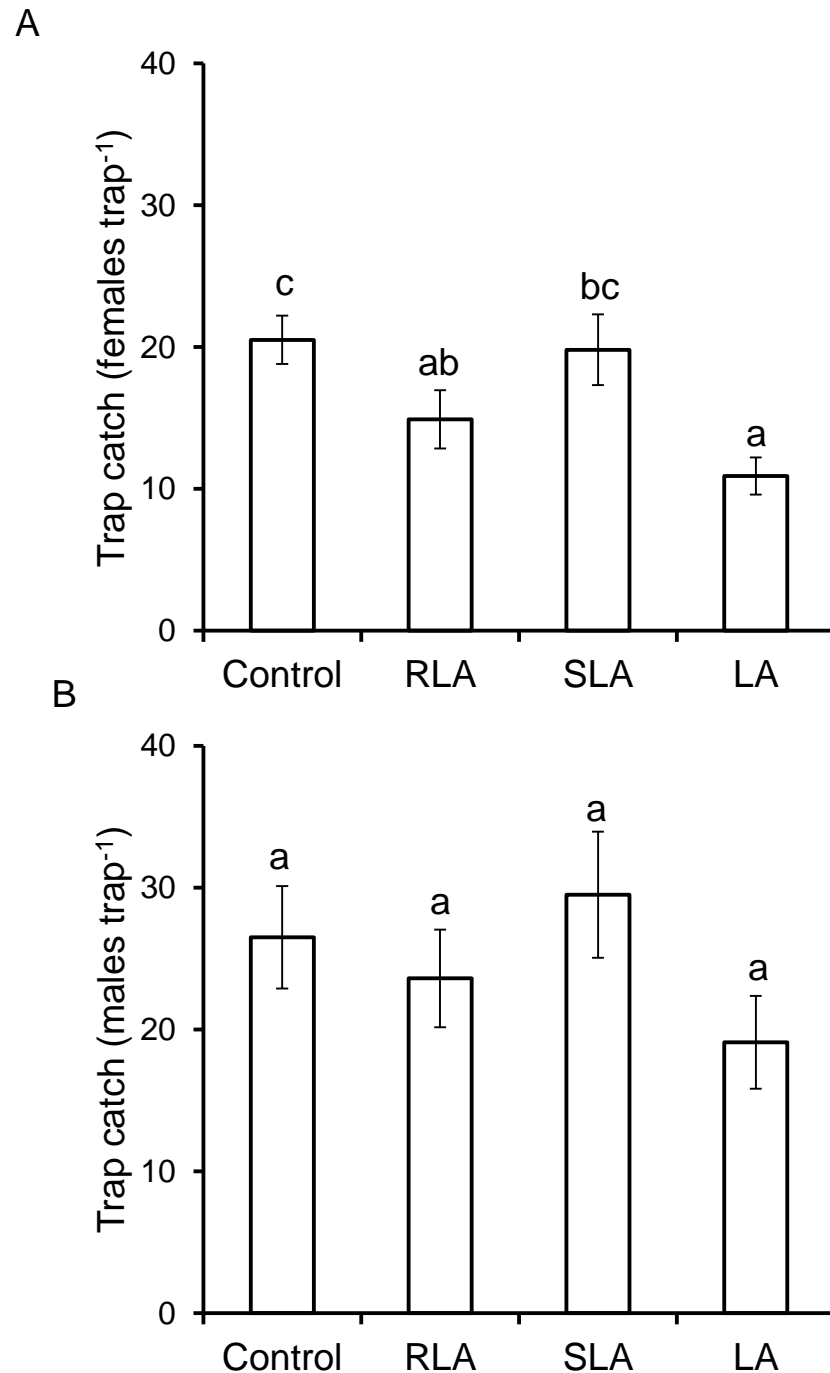


Figure 5.9. The mean number \pm SEM of adult *F. occidentalis* caught on yellow sticky traps with different chiral forms of lavandulyl acetate in vials, over 24 h in a pepper crop ($n = 20$) for (A) females ($F_{(3, 57)} = 9.1$, $P < 0.001$) and (B) males ($F_{(3, 57)} = 1.8$, $P = 0.15$). The release rate of RLA, SLA and LA was 0.4, 0.4 and 0.8 μg per day, respectively. RLA = (*R*)-lavandulyl acetate, SLA = (*S*)-lavandulyl acetate, LA = lavandulyl acetate. Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

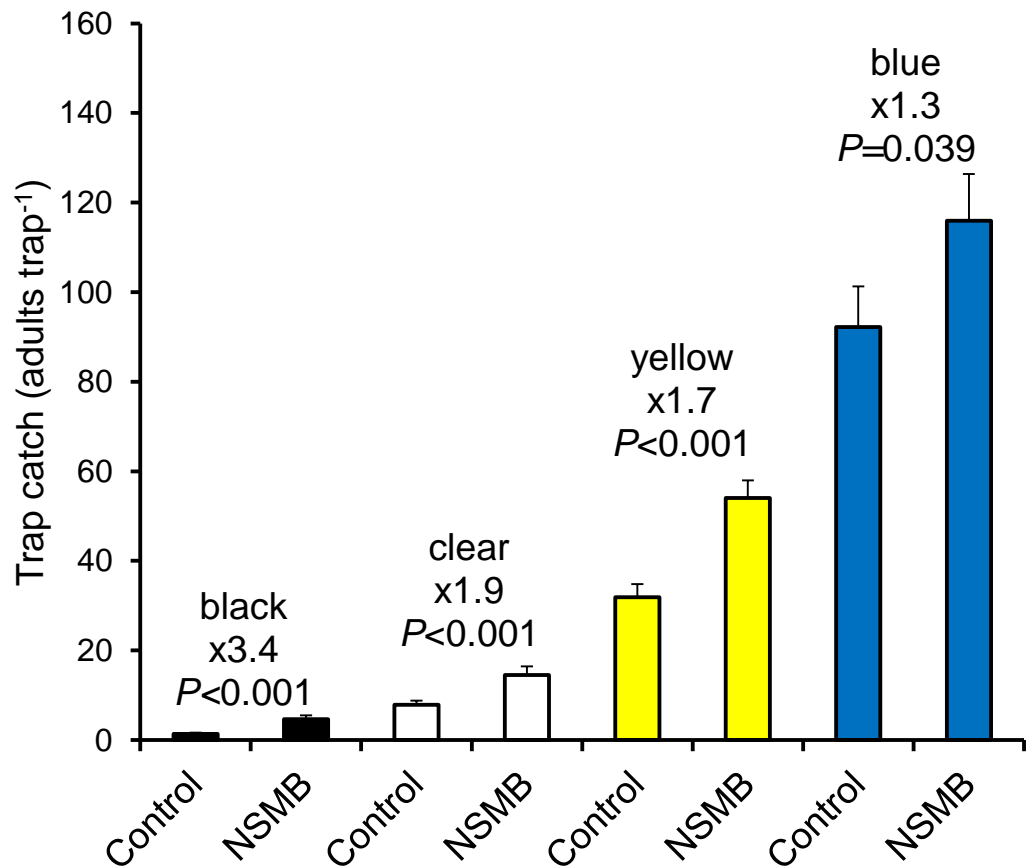


Figure 5.10. Mean catch + SEM of adult *F. occidentalis* on different coloured sticky traps with and without the aggregation pheromone neryl (*S*)-2-methylbutanoate on septa, over 24 h in a pepper crop (n = 19). Pair-wise comparisons between trap catch on control and pheromone treated traps for each trap colour are shown by *P* values and factor of increase. NSMB = neryl (*S*)-2-methylbutanoate. The release rate of NSMB was about 0.4 µg per day. Analysis was on transformed data whilst the charts show untransformed data.

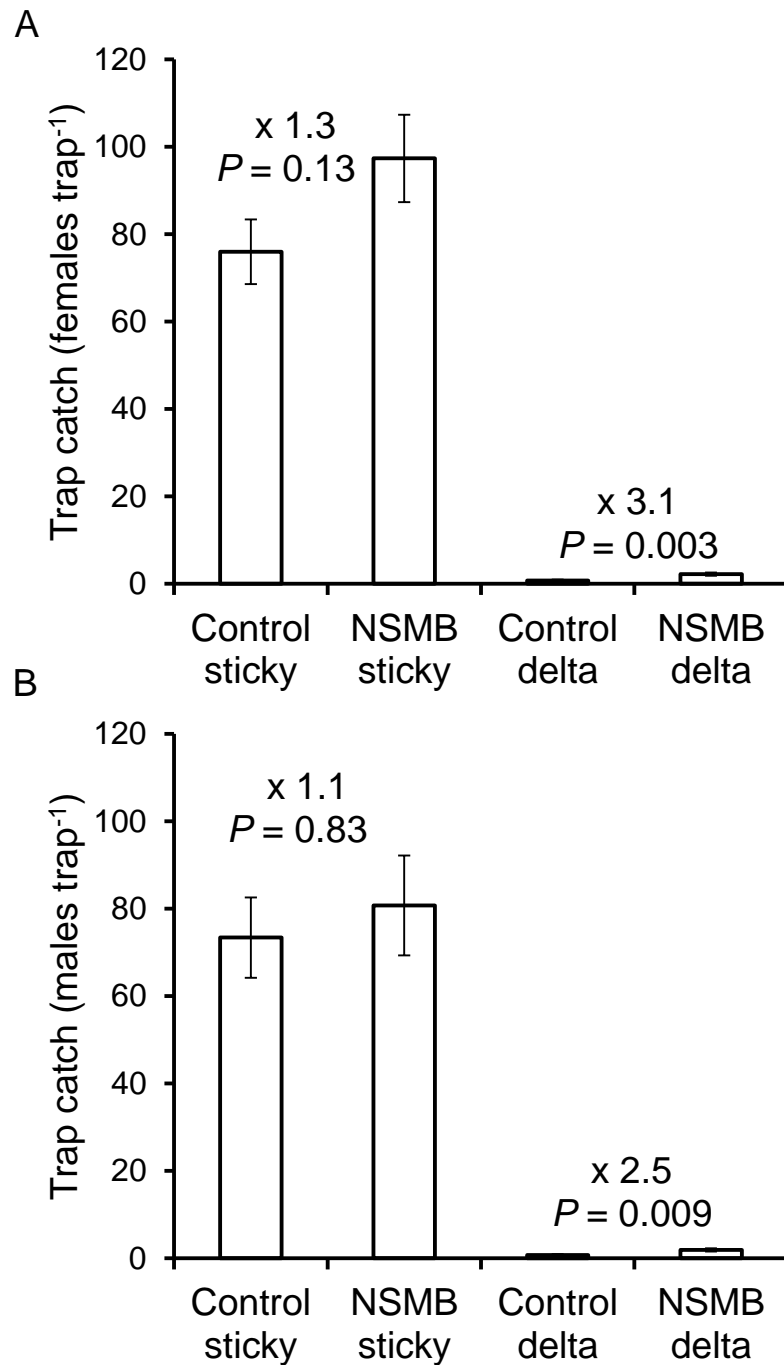


Figure 5.11. The mean catch \pm SEM of adult *F. occidentalis* on blue sticky traps alone, or blue sticky traps inserted into brown delta traps, with and without the aggregation pheromone neryl (*S*)-2-methylbutanoate (NSMB) on septa, over 24 h in a pepper crop ($n = 20$) for (A) females and (B) males. Pair-wise comparisons between trap catch on control and pheromone treated traps for each trap colour are shown by P values and factor of increase. The release rate of NSMB was about 0.4 μg per day. Analysis was on transformed data whilst the charts show untransformed data.

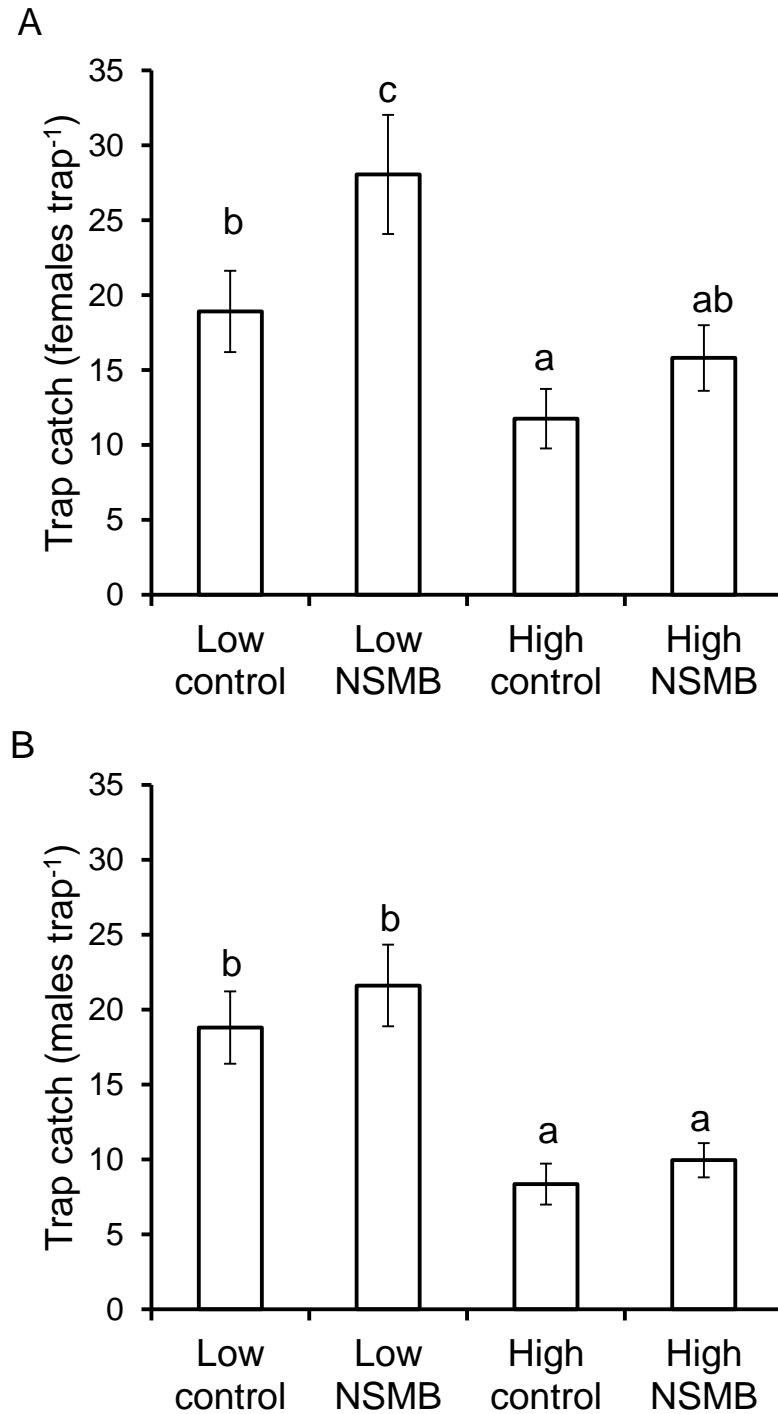


Figure 5.12. The mean number \pm SEM of adult *F. occidentalis* caught on yellow sticky traps at 20 cm (low) and 45 cm (high) above the crop, with and without the aggregation pheromone neryl (*S*)-2-methylbutanoate on septa, over 24 h in a pepper crop ($n = 20$) for (A) females ($F_{(3, 57)} = 14.6$, $P < 0.001$) and (B) males ($F_{(3, 57)} = 24.4$, $P < 0.001$). NSMB = neryl (*S*)-2-methylbutanoate. The release rate of NSMB was about 0.4 μg per day. Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

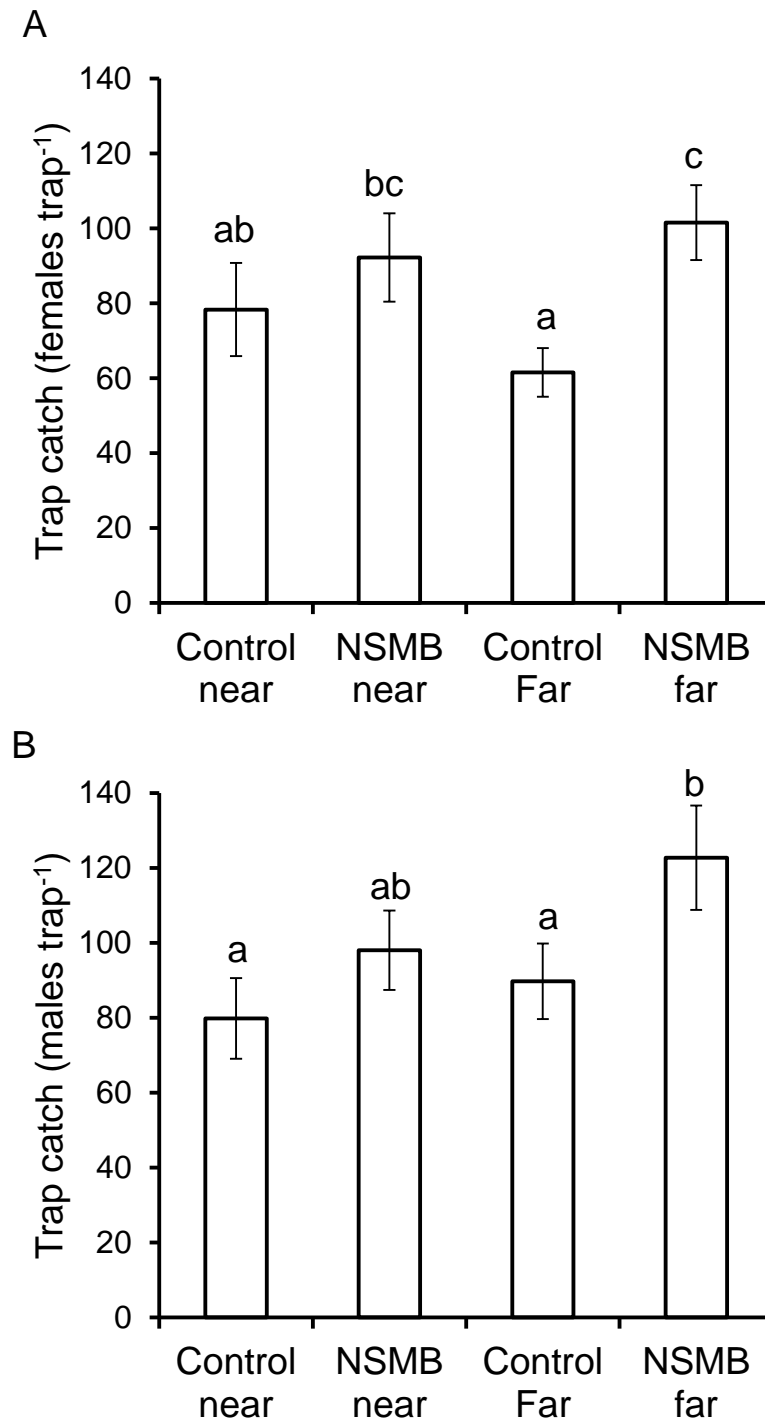


Figure 5.13. The mean catch \pm SEM of adult *F. occidentalis* on blue sticky traps at spacing of 1.2 m (near) and 6 m (far), with and without the aggregation pheromone neryl (*S*)-2-methylbutanoate on septa, over 24 h in a pepper crop ($n = 20$) for (A) females ($F_{(3, 57)} = 8.5$, $P < 0.001$) and (B) males ($F_{(3, 57)} = 7.1$, $P < 0.001$). NSMB = neryl (*S*)-2-methylbutanoate. The release rate of NSMB was about 0.4 μg per day. Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

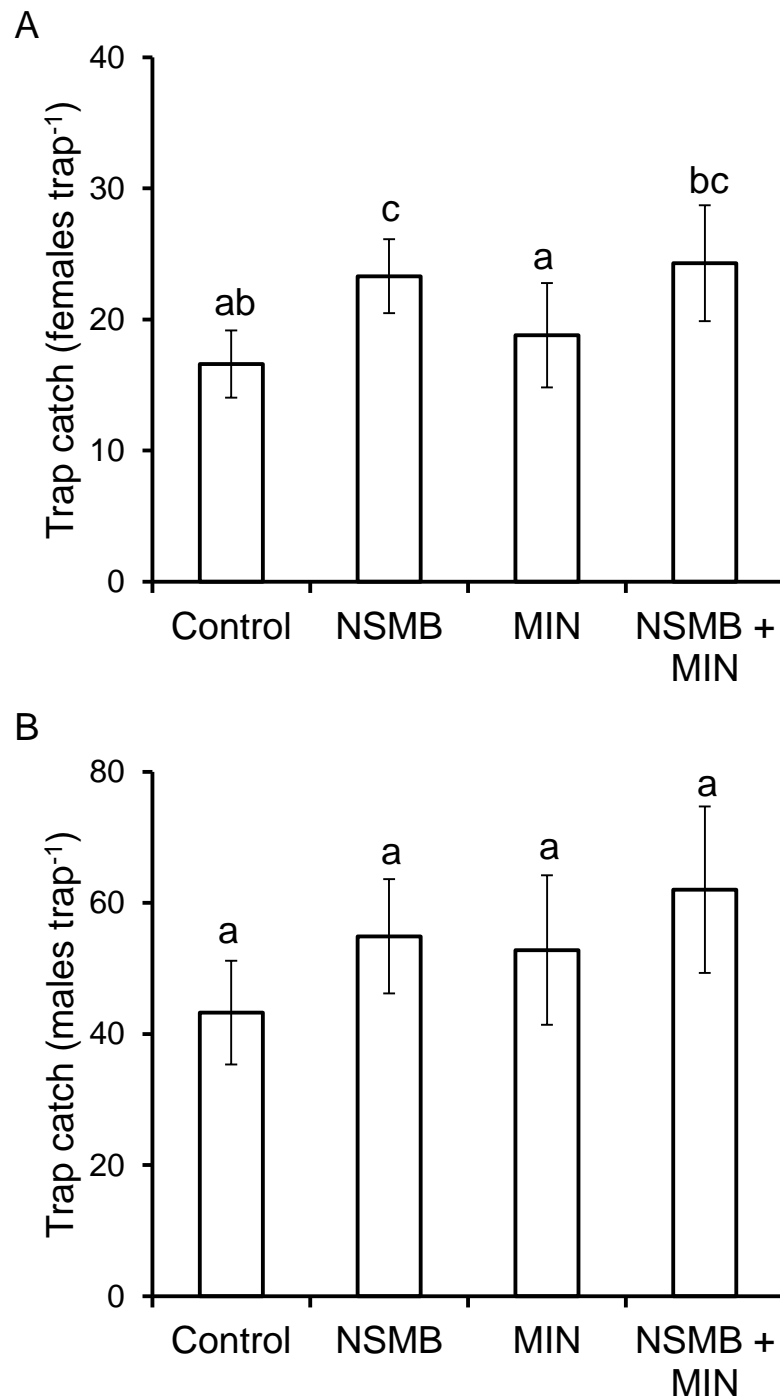


Figure 5.14. The mean catch \pm SEM of adult *F. occidentalis* caught on yellow sticky traps with neryl (*S*)-2-methylbutanoate on septa and methyl isonicotinate in sachets, over 24 h in a pepper crop ($n = 20$) for (A) females ($F_{(3, 57)} = 6.4$, $P = 0.001$) and (B) males ($F_{(3, 57)} = 2.1$, $P = 0.12$). NSMB = neryl (*S*)-2-methylbutanoate, MIN = methyl isonicotinate. The release rates of NSMB and MIN were about 0.4 μ g and 12.8 mg per day, respectively. Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

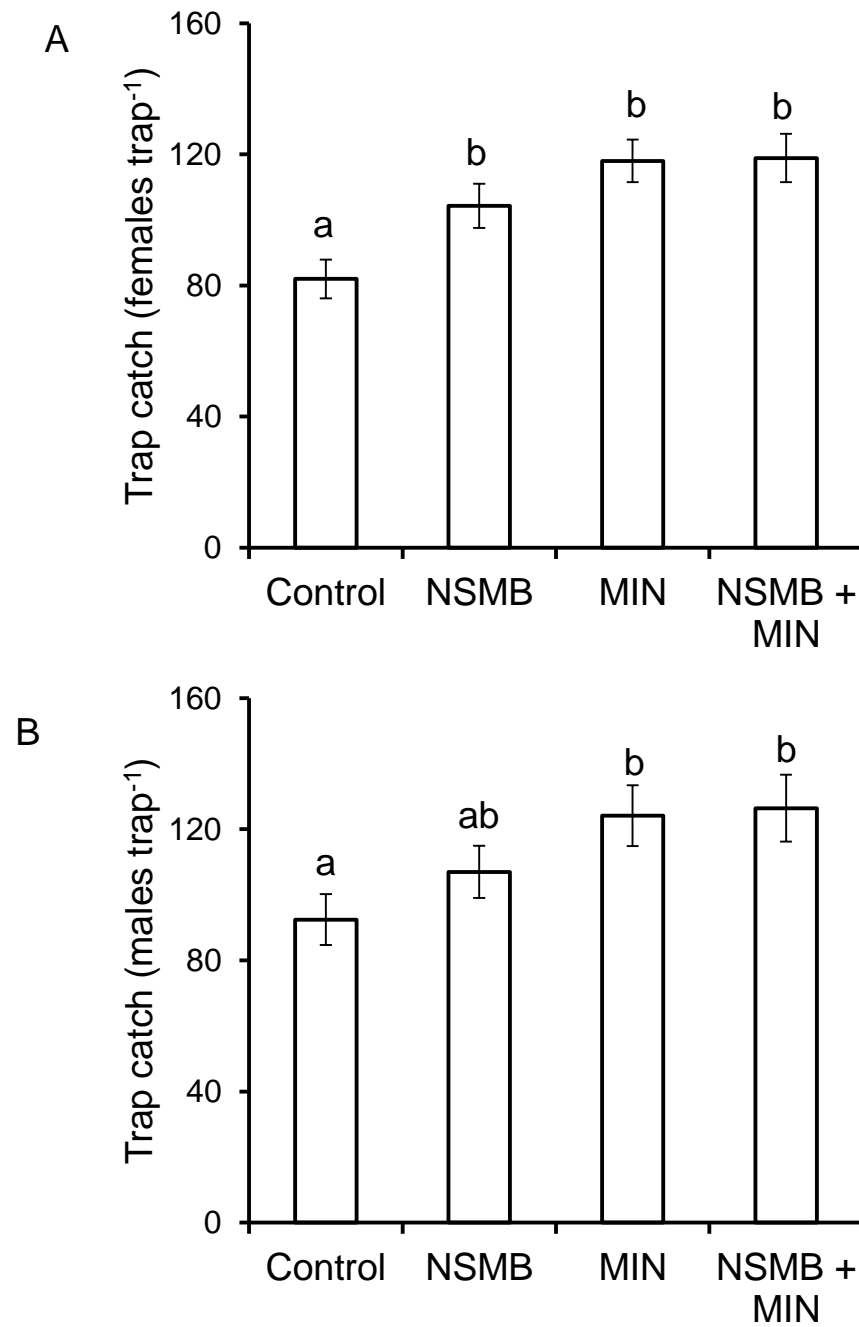


Figure 5.15. The mean catch \pm SEM of adult *F. occidentalis* on blue sticky traps with neryl (*S*)-2-methylbutanoate on septa and methyl isonicotinate in sachets, over 8 h in a strawberry crop ($n = 18$) for (A) females ($F_{(3, 51)} = 19.7$, $P < 0.001$) and (B) males ($F_{(3, 51)} = 10.8$, $P < 0.001$). NSMB = neryl (*S*)-2-methylbutanoate, MIN = methyl isonicotinate. The release rates of NSMB and MIN were about 0.4 μ g and 12.8 mg per day, respectively. Analysis was on transformed data whilst the charts show untransformed data. Means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

Chapter 6

Can mass trapping reduce thrips damage and is it economically viable?

6.1. Introduction

Mass trapping of insect pests is used routinely on more than 10 million hectares of commercial crops around the world, predominantly against Lepidoptera, Coleoptera, Diptera and Hemiptera (Witzgall *et al.*, 2010). A variety of lures are used to attract them, including food, colour, kairomones and pheromones, either alone or in combination (Jones, 1998). Interest in pheromone mass trapping has increased because traps can be species-specific, which reduces the impact on non-target species and because pheromones are active at very low concentrations and do not need to be sprayed directly onto a crop, which is safer for the environment than chemical insecticides. This gives a sustainable pest control strategy that can be integrated with the biologically-based programmes increasingly used. The greatest success has been against pest species that occur at low densities, have limited host ranges, long generation times, isolated populations and low mobility (El-Sayed *et al.*, 2006). In contrast, mass trapping is not widely used commercially against thysanopteran pests, which are typically polyphagous, have high population densities, short life cycles and rapid population increases (Kirk, 1997b). Thrips are difficult to control with trapping, as huge numbers need to be caught to make an impact on a population (Kawai & Kitamura, 1990). High densities of sticky traps have reduced *Thrips palmi* numbers in pepper and aubergine in Japan (Kawai, 1990) and *Frankliniella intonsa* in strawberry (Lim & Mainali, 2009) and pepper (Lim *et al.*, 2013) in South Korea, but no assessment of crop damage was done in these studies, so there was no evidence of economic viability. Natwick *et al.* (2007) found a negative correlation between trap catch and numbers of both *Frankliniella occidentalis* and *Thrips tabaci* on lettuce plants, suggesting that mass trapping could cause population reduction. Sticky traps failed to prevent damage from *T. tabaci* in onion (Trdan *et al.*, 2005).

This chapter examines the possibility of using mass trapping, as part of an integrated pest management (IPM) programme, against *F. occidentalis* in semi-protected strawberry. Scents and colours are used by flower-inhabiting thrips to locate flowers (Kirk, 1984; Terry, 1997) and both are utilised to increase trap catch for the monitoring or control of thrips. The best trap type and colour for mass trapping will depend on cost and practicality as well as the prevalence of other economically important pest and beneficial species in the crop. *Frankliniella occidentalis* are most attracted to a specific shade of light blue, containing a slight tint of white, with a peak in reflectance around 450 nm (blue), low reflectance at 350 nm (UV) and low reflectance at 600 nm (yellow-orange) (Brødsgaard, 1989; Vernon & Gillespie, 1990). A peak at 750 nm (red) is also present, but this is not considered relevant to trap catch as *F. occidentalis* is dichromatic, having two photoreceptors that respond in electroretinograms to peaks (λ_{\max}) at about 365 nm (UV) and about 540 nm (green) but do not respond to wavelengths above about 650 nm (Matteson *et al.*, 1992). White and yellow traps are also attractive, but generally to a lesser extent, while there is little or no attraction to black, green, red and clear traps (Brødsgaard, 1989; Broughton & Harrison, 2012; Sampson *et al.*, 2012). Trap catches decrease with increasing UV reflectance, so adding UV reflectance to the most attractive light blue traps reduces trap catch below that of standard non-UV white traps, while changing the blue shade (in a non-UV trap) to a darker blue results in a similar trap catch to the standard non-UV white (Vernon & Gillespie, 1990). In addition, trap catch increases with colour brightness (% reflectance) (Vernon & Gillespie, 1990; Matteson & Terry, 1992) so a bright light of a less attractive colour can be more attractive than a dull light of the most attractive colour, and *F. occidentalis* trap catch can be increased by the addition of blue light-emitting diodes (LEDs) (Chen *et al.*, 2004b). Both male and female *F. occidentalis* have a similar response to the colour of traps, but higher numbers of males are trapped during swarming (Matteson & Terry, 1992). In a few studies, white (Moffitt, 1964; Yudin *et al.*, 1987; Hoddle *et al.*, 2002) or yellow (Cho *et al.*, 1995b) traps have caught more *F. occidentalis* than blue traps. Hoddle *et al.* (2002) suggest that white may be more attractive than blue traps in outdoor crops and blue traps more attractive than white in protected crops, but there is no experimental evidence for this as the same trap types were not tested in both cropping situations and the commercial blue traps available do not always match the most attractive blue wavelength. There is increasing use of photo-selective cladding on polytunnels, to improve plant quality and for plant protection,

including UV-absorbing, thermic, fluorescent and pigmented films (Antignus, 2000; Doukas & Payne, 2007; Johansen *et al.*, 2011). These films could affect the optical properties and efficacy of traps which has not been fully tested, but is not examined here as the films were not used on the polytunnels in this study.

Predators, parasitoids and pollinators also use visual and odour cues for host finding and it is important to know the effect of trap colour and scents on beneficial insects to ensure that essential pollinators or natural enemies are not removed by trapping. The ideal trap colour would confer a degree of specificity, catching pest species without disrupting economically important beneficial species. In UK strawberry, key pollinators include honey bees (*Apis mellifera*), buff-tailed bumble bees (*Bombus terrestris*) and hoverflies (Syrphidae) (Garratt, 2012), which are essential for maintaining good fruit set and yield. Natural enemies such as *Orius* spp. and parasitic wasps (*Aphidius*, *Aphelinus* and *Praon* spp) are released for thrips and aphid control respectively (Sampson *et al.*, 2011) and augment naturally occurring predators, such as *Anthocoris nemorum* (Anthocoridae), hoverflies (Syrphidae), ladybirds (Coccinellidae), lacewings (e.g. *Chrysoperla* spp.) and *Aeolothrips intermedius* (Aelothripidae). Predatory mites, such as *Phytoseiulus persimilis* and *Neoseiulus cucumeris*, are used extensively for spider mite and thrips control in strawberry (see Chapter 1), but are unlikely to be affected greatly by sticky traps, as they do not fly. A relationship between ecological groups and colour attraction allows some prediction of trap catches to be made (Kirk, 1984; Kelber, 2001). In contrast to *F. occidentalis*, most phytophagous insect species are trichromatic, having three photoreceptors that respond to peaks (λ_{max}) at about 350 nm (UV), 440 nm (blue) and 530 nm (green), and yellow and white traps typically attract the widest range of leaf-feeding species, although flower-feeding species may also be attracted to the flower colour of their host plant (Prokopy & Owens, 1983; Kirk, 1984). Therefore some of the literature appears contradictory and cannot be fully interpreted without reference to the colour preferences of specific species and reflectance spectrum of the traps tested, which are not always available. More honey bees and solitary bees have been found on white traps than on blue traps, yet more bumble bees were found on blue traps (Clare *et al.*, 2000). Pollen feeding insects are usually attracted to yellow traps and hoverflies generally have a strong preference for yellow (Finch, 1992; Sutherland *et al.*, 1999; Laubertie *et al.*, 2006), yet there are some records of higher trap catch of hoverflies on blue traps than on white or yellow traps (Chen *et al.*, 2004a; Broughton & Harrison, 2012). *Orius* spp. also feed on

pollen and show attraction to flowers (Hansen *et al.*, 2003a), their own pheromones (Aldrich *et al.*, 2007) and plant volatiles emitted from infested plants (Venzon *et al.*, 1999). *Orius* spp. are recorded on blue, yellow and white traps (Boone, 1999; Atakan & Bayram, 2011), although sticky traps have been used extensively for monitoring of thrips without having any apparent negative impact on *Orius* populations. Yellow traps have caught more parasitic wasps than blue traps in citrus (Moreno *et al.*, 1984). Lacewings (*Chrysoperla carnea*) were more frequent on clear traps than on a range of colours (including blue, white and yellow) in alfalfa (Blackmer *et al.*, 2008), yet more *Mallada* spp. were caught on blue traps than on white traps in Australian orchards (Broughton & Harrison, 2012). *Aeolothrips* spp. have been recorded on blue and yellow sticky traps (Sampson *et al.*, 2012) and *Aeolothrips intermedius* shows a colour preference similar to *F. occidentalis* (Kirk, 1984). The pest species *Thrips major* and *Lygus rugulipennis* were present on strawberry plants in the study described in this chapter. *Thrips major* is attracted to both white and blue traps (Kirk, 1984) and *Lygus* spp are attracted to white traps (Blackmer *et al.*, 2008). Full testing of the impact of mass trapping on beneficial and secondary pest species was beyond the scope of this study, however, selected species were assessed on blue and white traps in UK strawberry, to give an indication of their prevalence and the possible impact of trapping on non-target species. The total number of insects caught on blue and white traps was also assessed as large numbers could contaminate the traps, reducing their efficiency.

A variety of trap types are used for thrips including water, sticky, light, suction, semiochemical traps and others (Lewis, 1997b). Blue sticky traps are most widely used to monitor *F. occidentalis* in commercial crops as they are relatively cost-effective and easy to manage (Brødsgaard, 1993b). Thrips trap catch is affected by trap size, shape and contrast with background, increasing with area of trap and length of perimeter (Kirk, 1987; Vernon & Gillespie, 1995; Chen *et al.*, 2006). Mainali and Lim (2008) and Lim and Mainali (2009) developed a daisy-shaped sticky trap for mass trapping and suggest that increased trap-catch of *F. intonsa* is because thrips recognise the flower shape, however the length of perimeter also affects trap catch (Carrizo, 2008), which is an alternative explanation that was not tested. Although traps with the greatest surface area would be most effective for mass trapping, they must be economic and placement must not interfere with crop management or plant growth. Traps catch most *F. occidentalis* when placed just above the crop canopy, close to flowers (Gillespie & Vernon, 1990; Laudonia & Viggiani,

1998), but deployment of a large number or surface area of sticky traps immediately above the strawberry beds is not practical in commercial crops, as the traps would interfere with crop work (picking and spraying), so two experiments in this chapter tested trap position in strawberry. Individual thrips have been observed to escape from sticky traps (Kirk, pers. com., 2011), especially during cooler periods when the glue loses some tackiness. In addition the efficiency of traps declines as they become contaminated with dirt and insect bodies. The rate of escape and decline in efficacy was tested, to give an indication of the possible benefit of replacing sticky traps.

Various scents, including *para*-anisaldehyde, methyl isonicotinate, and the *F. occidentalis* aggregation pheromone, neryl (*S*)-2-methylbutanoate, are known to increase thrips trap catch by similar amounts (Hamilton *et al.*, 2005; Teulon *et al.*, 2007b) (see also Chapter 5). These offer an opportunity for enhanced mass trapping that has not been tested. The pheromone is currently used for precision monitoring (Thripline_{ams}, Syngenta Bioline, Clacton, UK), but does not have EU registration as a control. Plant volatiles and their analogues have a broader spectrum of activity, increasing trap catch of some other pest and beneficial species, whereas the aggregation pheromone is generally more specific to *F. occidentalis* and did not significantly affect trap catch of the predator *Orius laevigatus* in a Spanish pepper crop (Broughton & Harrison, 2012; Sampson *et al.*, 2012). However, the aggregation pheromone is attractive to a North American species, *Orius insidiosus* (Waite, 2012). It could be that *O. insidiosus* has adapted to use the *F. occidentalis* aggregation pheromone as a kairomone in its native North America. Further studies are required to test the response of different *Orius* species to the *F. occidentalis* aggregation pheromone, as natural enemies are often the most important control method available to growers and it is important that mass trapping of the pest does not disrupt biological control.

The overall aim of this chapter was to identify a practical and cost-effective method of mass trapping *F. occidentalis* in semi-protected strawberry. Specific aims were to:

- (1) test the effect of trap colour (blue or white) on trap catch of *F. occidentalis*, other pest species and economically important beneficial insects in strawberry;
- (2) test the extent to which thrips escape from sticky traps;
- (3) test whether sticky traps decline in efficiency through time;
- (4) test the effect of trap height and orientation on trap catch;

- (5) identify a practical and effective placement of sticky roller traps in commercial crops;
- (6) determine whether traps are effective throughout the growing season;
- (7) test whether mass trapping with blue sticky roller traps catches sufficient *F. occidentalis* to reduce fruit damage in semi-protected strawberry;
- (8) test whether there is added benefit of the aggregation pheromone for mass trapping with blue sticky roller traps;
- (9) determine whether mass trapping is economically viable in UK semi-protected strawberry.

6.2. Materials and Methods

A series of experiments was carried out in commercial everbearer strawberry (*Fragaria x ananassa*) crops, that were continuously flowering from April to October. The crops were grown under semi-protected polytunnels in the West Midlands region of the UK (Figure 2.1). The polytunnels were open-sided and in place from late March through to November. Details of the fields and growing systems used are summarised in Chapter 2 (Table 2.1). Experiments were carried out in fields where the thrips species and their distribution was largely known, following the routine monitoring, distribution and damage studies detailed in Chapters 3 and 4. The fields were selected for their infestation level and thrips species composition. The thrips population in the fields selected for the trap position and trap stickiness experiments consisted of >90% *F. occidentalis* and there were few other pests, which allowed testing with minimal contamination of traps with other insect species. A field with a greater diversity of species was selected for the trap colour experiment so that the impact on non-target species could be tested. Within the fields there were local gradients in thrips density (see Chapter 3). As a result there was considerable background variation that would tend to obscure real treatment effects unless they were large or the experiments well replicated. As far as possible, the trap colour, efficiency and trap position experiments were placed in areas of the fields where the thrips density was similar and the experimental design was blocked to reduce these effects, however there was still much variation that could not be removed statistically. Fewer blocks were possible in the three mass trapping experiments because of the large plot size required (see 6.2.6 for the experimental design of the mass trapping experiments).

Initial experiments investigated trap colour, decline-rate and position in the crop, and the following methods were common to these unless stated otherwise, and will not be repeated in each section. Methods used for testing the efficiency of trapping through the season and for the mass trapping experiments are shown separately. Blue sticky monitoring traps were used (10 cm high by 25 cm wide) from Russell IPM (Impact trap, Russell IPM Ltd, Deeside, UK) or Syngenta Bioline (Takitrapline b, Syngenta Bioline Ltd, Clacton, UK), which are widely used for monitoring *F. occidentalis*. White traps (Russell IPM Ltd) were also used for the trap colour experiment as some white traps have caught more *F. occidentalis* than some blue traps (Hoddle *et al.*, 2002). Two different suppliers of blue monitoring traps were used as both companies were partners in the project. Both trap types matched to the same shade of light blue in Pantone colour charts (see 6.3), but no direct comparison of the efficacy of the traps was made. Only one trap type was used within a single experiment.

The spectral reflectance (300-700 nm) of the different blue and white sticky traps used during this study was measured by Dr Sarah Arnold (NRI, Chatham, UK). It was measured using a spectrophotometer (AvaSpec-2048, Avantes, Apeldoorn, NL) with a Deuterium-Halogen light source (AvaLight-DH-S-BAL, Avantes, Apeldoorn, NL). The spectra were calibrated relative to a barium sulphate white standard (WS-2, Avantes, Apeldoorn, NL), using a fine probe (FCR-7UV200-2-1.5 x 100, Avantes, Apeldoorn, NL) at 45° to the stimulus surface using a light shade probe holder (Knight Photonics, Leatherhead, UK) (Chittka & Kevan, 2005). Three measurements were made on each type of blue sticky monitoring traps from Syngenta Bioline and Russell IPM, on an area without adhesive and three on an area with adhesive. Three measurements were also made on an area with adhesive on the white traps. The white traps had been specifically made by Russell IPM for the experiment and there were no areas without adhesive to test. Three measurements were also made on an area without adhesive on a roller trap (Optiroll), but no test was done on the adhesive area, as the available traps had been used in the experiments. To measure over the sticky part, the sticky trap was covered with a thin piece of cellophane which does not have any significant absorbance. Each trap colour was also matched by eye to Pantone colour charts.

At the start of each experiment, a sample of 20 plants and 20 mid-aged flowers (see Chapter 2) was taken from the experimental area, with the sample plants spaced regularly over the trial area. The number of flowers per plant and number of adult thrips per

medium-aged flower was counted by eye using a x7 head lens (optiVISOR). The sampled flowers were placed in 70% alcohol and the adult thrips were counted and the *F. occidentalis* separated from the other thrips species, using the methods described in Chapter 2. These pooled samples were used to calculate the percentage of *F. occidentalis* in the eye counts (section 6.2.8).

Sticky monitoring traps were stuck vertically (landscape orientation) onto bamboo canes (60 cm), which were pushed into the ground so that the bottom edge of each trap was about 10 cm above the crop canopy with about 1 cm of cane extending above the traps. Traps were secured with a rubber band (size 33, Censtretch, Rochester, UK) that was placed over the cane, twisted over the trap, then slotted back over the cane. A landscape orientation was used so that the traps would fit under a tractor during crop work, as traps in portrait orientation were too high and were knocked over. Sticky monitoring traps faced south, unless stated otherwise, to avoid any bias that might occur through wind or sun direction. Traps were placed at least 20 m inside from the ends of the polytunnels to reduce edge effects, as the ends of the polytunnels were cooled by the outside air, have more direct sunlight, or could be affected by the thrips present in the weeds along the field margins. Unless stated otherwise, the traps were spaced 2.2 m apart. A spacing of 2.2 m was used, rather than the 4.8 m spacing used for the experiments in Chapter 5, to reduce the background variability in thrips numbers. This spacing was the same spacing as the polytunnel legs which gave an easy reference for placing the traps but also ensured that every trap was in the same position relative to the polytunnel cladding.

At the end of each experiment, traps were removed from the crop, wrapped separately in clear, thin polythene and stored in a freezer. Trap catches were counted under a binocular microscope in the Keele laboratory, as described in Chapter 2. Aeolothripid (with broad wings) and Phlaeothripid thrips (with elongated last abdominal segment) were excluded from the counts unless stated otherwise as these can be predators and are not considered pests of strawberry. With the exception of the trap colour experiment (section 6.2.1) the flower samples were used to indicate the species present on the traps. Although it is probable that the blue sticky traps were selectively more attractive to *F. occidentalis* than to some of the other thrips species present (Kirk, 1984), this had a minimal effect on the total trap catch because of the high proportion of *F. occidentalis* present (e.g. the percentage of *F. occidentalis* may be 95% in flowers and 96% on traps, C. Sampson, unpublished data, 2012). The species found in the strawberry flowers were therefore

considered broadly indicative of those on the traps. For the rest of this chapter thrips refers to species in the Thripidae family.

A data logger (EL-USB-1, Lascar Electronics, Salisbury, UK) was placed in a white delta trap (273 mm length, 130 mm height, œcos, Kimpton, UK) to shade it from the sun and was placed in the crop canopy, in a central area of each experiment, to record temperature and humidity.

6.2.1. What is the effect of trap colour on trap catch of pest and beneficial insects?

To select the best trap colour for mass trapping, the trap catches of selected pest and beneficial species was compared, on blue and white traps in a semi-protected strawberry crop (cv. Camarillo, field 3, Table 2.1). Blue and white sticky monitoring traps (Impact traps, Russell IPM Ltd) were used as they are the most attractive colours to *F. occidentalis* (see 6.1). The white traps were made up specifically for the experiment in small sheets by Russell IPM. Both trap colours were cut to the same size (16 cm wide x 10 cm high) to allow direct comparison. The two trap colours were paired and laid out in a randomised complete block design on 6 August 2012. There were 15 blocks and one replicate per block with 2.2 m between all traps (within and between blocks). All the traps were placed in two adjacent strawberry beds in a row along the centre of a single tunnel. Treatments were:

- Blue sticky monitoring traps (16 cm wide x 10 cm high);
- White sticky monitoring traps (16 cm wide x 10 cm high).

The traps were removed after 96 h in the crop, wrapped separately in clear polythene and stored in a freezer. Initially a few traps (five of each colour) were scanned rapidly under a binocular microscope to see which pest and beneficial species had been caught. The majority of insects on the traps were thrips, which were present in high numbers, so these were sub-sampled. Thrips were removed from four sections of each trap using the methods shown in section 2.4, and then thrips from the four sections were pooled together in 70% alcohol. Each section was 1 cm wide and 1 cm in from the top or bottom of the trap, repeated on both sides, which totalled 12.5% of the total trap surface. *Frankliniella occidentalis* and *Thrips major* were the most numerous thrips species on the traps and these were separated from each other and from other thrips species by eye (see Chapter 2)

and were counted under a binocular microscope whilst in alcohol. Then sub-samples of thrips were placed on microscope slides (see Chapter 2) to confirm the identification under a compound microscope. Confirmation of the identification (on slides) was carried out of 50 randomly selected specimens considered to be *F. occidentalis*, using a grid square on the bottom of the petri-dish, 50 randomly selected specimens considered to be *T. major* and of all the remaining thrips that were neither of these species.

Another important pest species on strawberry, the European tarnished plant bug, *Lygus rugulipennis* was caught on the traps. Whole trap counts of *Lygus* bugs were made, which were separated from other species of mirid bug (such as *Orius* spp. and *Anthocoris nemorum*) by their characteristic body shape and distinctive lighter coloured scutellum. One, randomly selected *L. rugulipennis* on each trap was examined under a binocular microscope to confirm the identification, based on the densely pubescent corium (n = 30 *Lygus* bugs) (Nau, 2004). Whole trap counts of beneficial insects were made. These included the predatory thrips *Aeolothrips intermedius*, identified by its broad, black and white-striped wing and pale wing-tip vein; bees, including bumble bees, solitary bees and honey bees, which were identified as bees (not to species) by two pairs of wings, large, hairy body and colour pattern of their hairs (Edwards & Jenner, 2009); hoverflies (Syrphidae), which were identified by their brightly coloured patterns, single pair of wings and the presence of a spurious vein (vena spuria) found parallel to the forth longitudinal wing vein (Stubbs & Falk, 2002); other beneficial species were present in very low numbers, these included Coccinellidae, Anthocoridae and parasitic wasps. The beneficial insect data were combined for analysis because there were insufficient individuals of any one species. A count of the total number of insects per trap (excluding thrips) was carried out to give a measure of possible contamination of the traps for each of the two colours.

6.2.2. Can thrips escape from sticky traps?

Some thrips may escape from the traps. To determine whether thrips escape from sticky monitoring traps, traps were placed in a field with a moderate thrips population for a day, then moved to a nearby field with few thrips, after which half the traps were collected in and the other half left overnight in a field where few extra thrips would be caught. The trap catches of the two sets of traps were compared to test whether there had been a decline in trap catch overnight. Further traps were placed in the field with few thrips overnight to determine the overnight trap catch. The experiment was carried out in the field rather than

in the laboratory, as this allowed thrips to fly and land on the traps naturally at field temperatures, which could affect escape rate. The traps that were only *in situ* during the day were moved to the new field before collection to ensure that any reduction in thrips numbers was not due to them being moved. A reduction in thrips trap catch overnight would suggest that thrips are escaping from the traps. If a significant number of thrips are escaping from traps, it could have implications for the trap design or the frequency of replacement (see 6.2.2).

Escape from sticky monitoring traps was tested in two semi-protected strawberry crops (cv. Finesse, fields 5 and 7, Table 2.1) using cut-down blue sticky monitoring traps (10 cm x 5 cm, Impact traps) laid out in a randomised complete block on 6 August 2012. Trap size was reduced because the thrips population was high, so sufficient thrips were caught on small traps to identify a difference between treatments. There were 10 blocks and one replicate per block with 2.2 m between all traps (within and between blocks). Traps were placed in a strawberry bed row along the centre of a single tunnel. Treatments were:

- Day traps: Traps were placed in field 7 on 6 August from 9.00 h to 16.30 h, then moved to field 5 before being collected at 17.00 h (day trap catch in a field with moderate numbers of thrips);
- Day and night traps: Traps were placed in field 7 from 9.00 h to 16.30 h on 6 August, then moved to field 5 where they were in place from 17.00 h on 6 August until 8.00 h on 7 August (day trap catch plus overnight in a field with few thrips);
- Night traps: Traps were placed in field 5 from 17.00 h on 6 August 2012 to 8.00 h on 7 August 2012 (overnight in a field with few thrips).

In addition to counts of the total trap catch (see 6.2), the number of thrips touching the edge of each trap was counted on the traps that were *in situ* during the day and for 23 h, as this could help provide information about how the thrips are escaping. It is possible that thrips are moving to the edge of the trap and using the edge as a lever to escape the trap glue.

6.2.3. Do traps decrease in efficiency through time?

Sticky traps may decline in efficiency through time because they become contaminated with dirt and insects or because the glue washes off or becomes less sticky.

If this is the case, then proportionally more thrips would be caught when traps are first deployed and progressively fewer thrips thereafter. This was tested by leaving traps out for a total of four days, but replacing the traps within each treatment at different frequencies. If there is no decline in efficiency, then the total trap catch would be about the same for all treatments, but if there is a decline then trap-catch would increase with the number of trap replacements, so the traps would have to be replaced frequently for maximum effect.

The effect of time on the efficiency of sticky monitoring traps was tested in a commercial semi-protected strawberry crop (cv. Finesse, field 7, Table 2.1) using blue sticky monitoring traps (25 cm x 10 cm, Impact traps) laid out in a randomised complete block on 6 August 2012. There were 10 blocks and one replicate per block with 2.2 m between all traps (within and between blocks). Traps were placed in a strawberry bed row along the centre of a single tunnel. Treatments were:

- Traps that were replaced daily for four days (4 traps, each for 24 hours);
- Traps that were replaced after two days (2 traps, each for 48 hours);
- Traps that remained in place for four days (1 trap for 96 hours).

6.2.4. Trap placement in commercial strawberry

The following two experiments sought to optimise the placement of traps for mass trapping in strawberry, without interfering with crop work or plant growth.

6.2.4.1. Does trap height and orientation affect trap catch?

Sticky traps were placed at different heights and orientations above a strawberry crop and the trap catches were compared to determine which trap positions resulted in the highest trap catch. This information was used to help decide how traps should be placed for maximum effect for mass trapping (see 6.2.6).

The effect of trap height and orientation on trap catch was tested in a commercial semi-protected strawberry crop (cv. Albion, field 1, Table 2.1) using blue sticky monitoring traps (25 cm x 10 cm, Takitrap b) laid out in a randomised complete block design on 25 August 2011. There were 20 blocks, each in a different tunnel, and one replicate per block with 4.4 m between traps within a block and 13 m (two tunnels) between blocks. Treatments were:

- Vertical trap, landscape orientation, low (base 10 cm above the crop), south facing;
- Vertical trap, landscape orientation, low (base 10 cm above the crop), east facing;
- Vertical trap, portrait orientation, low (base 10 cm above the crop), east facing;
- Vertical trap, landscape orientation, high (base 30 cm above the crop), east facing;
- Horizontal trap, 10 cm above the crop.

The traps were suspended above the crop by sticking them onto bamboo garden canes that had been pushed into the ground and were secured with wooden clothes pegs. The horizontal trap was supported on bent wire curled around the bamboo cane. The traps were removed from the crop after 24 h, wrapped separately in clear polythene and stored in a freezer. The wind speed and direction was recorded by Mr S. Clarke from Manor Farm's weather station (Vantage Pro2, Davis, Hayward, USA), which is placed outside the strawberry tunnels in a central position on the farm.

6.2.4.2. Are traps effective when placed between the tunnels?

Placement of traps within strawberry beds could affect plant growth, disrupt crop work and make the traps vulnerable to damage from tractors, whilst placing them between the tunnels, in the area where the tunnel legs reach the ground, may be more practical and less disruptive for growers. The effect of placement on trap catch was tested by placing traps in strawberry beds and between the tunnels, then comparing the trap catch. If the trap catch is little affected by placing the traps between the tunnels, then that placement could be used for mass trapping (see 6.2.6).

The experiment was carried out in a commercial semi-protected strawberry crop (cv. Finesse, field 7, Table 2.1) using blue sticky monitoring traps (25 cm x 10 cm, Impact traps) laid out in a randomised complete block design on 6 August 2012. There were 18 blocks and one replicate per block with 3 m between traps within a block (the distance between the middle of the strawberry bed used and the area between the polytunnels where the tunnel legs reach the ground) and 6.5 m (1 tunnel) between blocks. Treatments were:

- Blue sticky trap (25 cm x 10 cm, Russell IPM) placed within the strawberry beds;
- Blue sticky trap (25 cm x 10 cm, Russell IPM) placed between the strawberry beds.

Traps were stuck vertically (landscape orientation) onto bamboo canes (60 cm) with the bottom edge of the traps about 10 cm above the crop within beds or 50 cm above the ground (at the same height above ground as the bed traps) between the strawberry beds, in

the area where the polytunnel legs reach the ground. The area between the polytunnels was 1 m wide, covered with straw mulch and there were few weeds. All traps were south facing. After four days the traps were removed from the crop, wrapped individually in clear polythene and stored in a freezer. Male and female thrips were counted separately on the traps and in the flower samples from the field.

6.2.5. Are traps effective throughout the growing season?

To determine the relative efficiency of trapping through a season, thrips population density on a strawberry crop and pheromone trap catches were sampled weekly. A “trapping efficiency index” was calculated each week by dividing the numbers of thrips per trap by the estimated numbers of thrips per m² to give a relative measure of the proportion of the population caught by trapping. Thus it can be used to indicate when trapping efficiency was high or low. This was tested in a semi-protected strawberry crop (cv. Camarillo, field 3, Table 2.1), which was monitored weekly from first flowering on 17 May (earlier flowers had been de-blossomed as is common commercial practice) to the end of cropping on 18 October 2011. The grower continued with his usual thrips control programme, which included releases of the predatory mite *Neoseiulus cucumeris* (Oudemans) (Acarina: Phytoseiidae) (about 100 per plant spread over the season) and two insecticide treatments with spinosad (Tracer, Landseer Ltd., Chelmsford, UK) on 15 and 30 July 2011.

The methods used for trapping and estimating the thrips population are detailed in section 3.2.1, Chapter 3 and will not be repeated here. Briefly, one blue sticky trap (25 cm x 10 cm, Impact trap, Russell IPM) with a pheromone lure (Thripline_{ams}, Syngenta Bioline Ltd) and 10 plants within 10 m of the trap were monitored weekly in each of two separate tunnels (n = 1 trap, 10 plants). The numbers of flowers and fruit per plant were counted and one flower and one white fruit were selected from each of the 10 plants and the numbers of thrips per plant part were counted (n = 10 flowers and 10 white fruit).

A simple estimate of the numbers of thrips per m² for the purpose of comparison was made by multiplying the mean numbers of adult thrips per flower by the numbers of open flowers on the 10 plants, then adding the mean numbers of adult thrips per white fruit multiplied by the numbers of fruit (all stages) on the 10 plants. Numbers of adult *F. occidentalis* typically increase as the fruit matures from green to white to red, with about twice as many adult thrips on red fruit compared to those on green fruit (Steiner &

Goodwin, 2005a), so an intermediate stage was used. Although this is an underestimate, as it does not include thrips on leaves or off the plant, it is a relative measure of the thrips population for looking at variation over time. In whole plant counts in different fields during the season, on the cultivar Camarillo, about 1% of *F. occidentalis* adults were found on strawberry leaves compared to flowers and fruit (see Chapter 4).

6.2.6. Can mass trapping reduce thrips numbers and fruit damage?

6.2.6.1. Pilot experiments in a first-year and a second-year crop

In these pilot experiments, a high density of traps with added plant volatile and aggregation pheromone were used to test whether mass trapping could reduce thrips numbers and fruit damage in UK strawberry. The trapping was not necessarily practical or economically viable. The experiments were set up in two crops concurrently: in a first-year crop with lower thrips numbers at the start of the season (cv. Camarillo, field 3, Table 2.1), and in a second-year crop with an established thrips population (cv. Camarillo, field 4, Table 2.1). Traps were used in addition to the grower's usual thrips control programme, which included releases of the predatory mite *N. cucumeris* (a total of 200-300 per plant spread between April and July); one insecticide treatment with spinosad (Tracer) in field 4 on 14 June 2012 and in field 3 on 15 August 2012; and one treatment with Naturalis (*Beauveria bassiana*) in field 4 on 10 July 2012. Blue sticky traps were used as they are known to be attractive to *F. occidentalis* and more specific to *F. occidentalis* than white traps (see 6.3.1). The roller traps were placed vertically, at flower height, between the tunnels, in the area between the strawberry beds where the tunnel legs reach the ground, to maximise the trap catch of female thrips (see 6.3.4). The plant volatile analogue, methyl isonicotinate and the aggregation pheromone neryl (*S*)-2-methylbutoanoate were added to maximise the trap catch (see Chapter 5). In each field there were four matched pairs of treated and untreated plots (Figure 6.1 A, B). Each plot was 17.6 m long and 6.5 m (one tunnel) wide, with 19.5 m (three tunnels) in between each pair. Plot length was determined by the length of available roller trap and the distance between tunnel legs, to which the traps were attached. The treatment position within the first pair was chosen randomly, but the treatment position for the rest of the pairs was alternated, as previous surveys had shown local gradients in both fields with higher thrips densities towards the top of the tunnels and because there was a heavily infested crop adjacent to the first-year crop. All plots were located at least 40 m in from the ends of the polytunnels to reduce edge effects.

In both crops, the tunnels were 6.5 m wide with four beds of strawberry per tunnel.

Treatments were:

- Control plots without any traps or lures;
- Treated plots with both traps and lures.

In treated plots, blue sticky roller traps (Optiroll, 100 m x 30 cm, cut to plot length, Russell IPM Ltd) were placed along each side of the plots in three, 4.4 m sections, with 2.2 m (distance between legs) between each section (Figure 6.2 A). When the traps were put up for the first time, gaps were left for practical reasons as I was putting up the traps on my own and used shorter lengths of trap, wrapping the ends around the posts to ensure the traps remained taut. The blue sticky roller traps were double-sided and placed down both sides of the treatment plots, clipped onto the polytunnel legs between strawberry beds using polytunnel securing clips (20 mm wide, 30 mm diameter) protected by a polythene strip, (approx. 30 mm x 80 mm) (Figure 6.2 B). The base of each trap was level with the crop canopy (approximately 30 cm above the ground). Within each treated plot, blue sticky monitoring traps (25 cm x 10 cm, Impact trap) were placed down the two middle strawberry beds at 2.2 m intervals (9 per bed). For extra secure fixing, the canes were pushed through a slit that was made in the middle of each trap (Figure 6.2 C). The sticky monitoring traps were not replaced if they were knocked over, to give an indication of the practicality of the method. The traps were put up on 23 April 2012 (1st year crop) and 19 April 2012 (2nd year crop) and were replaced on 27 June 2012 (both crops). When the roller traps were replaced in June, they were replaced in a continuous run along each side of the plot (17.6 m long), with two clips holding the trap in place at every leg. This was made possible by getting help from Mr Ron Knapper (Keele University) and Ms Zlatka Zapryanova (Manor Farm staff) to put the traps up.

The pheromone lures, each containing 30 µg neryl (*S*)-2-methylbutanoate (Thripline_{ams}, Syngenta Bioline) were placed in the string hole of each central trap and at 2.2 m intervals down the roller traps (Figure 6.2 C, D) (a total of 36 lures, 1080 µg pheromone). Each pheromone lure gave a release rate of about 0.4 µg neryl (*S*)-2-methylbutanoate per day at 27°C (NRI, see Chapter 5). A spacing of 2.2 m was adopted partly for convenience as the interval corresponded with the spacing of the polytunnel legs and partly because previous experiments had shown that the pheromone increases trap catch on traps that are at least 1.2 m away (see Chapter 5). Four polythene sachets (50 mm

x 50 mm x 120 µm thick), each containing 250 µl methyl isonicotinate (SigmaAldrich) were stapled onto traps (without puncturing the sachets) with one on each roller trap and one in each central bed (a total of four lures, 1000 µl of plant volatile). Each sachet gave a release rate of about 12.8 mg per day at 22°C (NRI, see Chapter 5). The spacing for the plant volatile lures was adopted to keep the total volume of pheromone and plant volatile per plot approximately equal based on the volume of the scents used in commercial lures.

An assessment of thrips numbers was made on 12 April 2012 (1st year crop) and 19 April 2012 (2nd year crop) before the traps were put up, then at approximately monthly intervals on 23 May, 21 June, 18 July, 13 August (1st year crop) and 16 May, 12 June, 11 July, 10 August (2nd year crop). On each assessment date, 40 medium-aged flowers and 20 fully swollen white fruit (once available) were sampled regularly from across each plot, excluding 2.2 m in from the ends to reduce edge effects. The assessment of white fruit enabled comparison of the same fruit stage between plots and dates, as red fruit of comparable ripeness was not always available following picking and the selective picking of undamaged red fruit would have biased red fruit samples. The numbers of adult thrips per flower and the numbers of seeds surrounded by bronzing per fruit were counted by eye in the field using a x7 head lens (optiVISOR) as eye counts were considered both effective and reliable and could be related in future to grower counts for monitoring (González-Zamora & Garcia-Marí, 2003) (see Chapter 2). Flowers were pooled and placed in 70% alcohol so that thrips could be extracted and identified to species. These flower counts were used to identify the percentage of *F. occidentalis* in the field (see 6.2.8). The numbers of flowers per plant were counted on 10 plants from the middle of the trial area on each assessment date.

To test the impact of trapping on thrips density, simple estimates of the numbers of adult thrips per plot (on each sample date) and thrips per roller trap were made, when the traps were changed and at the end of the experiment. Numbers of adult thrips per plot were estimated by multiplying the mean numbers of adult thrips per flower (n = 40 flowers) by the mean numbers of flowers per plant (n = 10 plants) and plants per plot (set by the planting density). Flower counts, although providing an underestimate of the thrips population, would account for over 74% of the adult *F. occidentalis* population in strawberry (see Chapter 4), so an underestimate but reasonably close. The numbers of thrips on roller traps were estimated by counting the total numbers of thrips on six randomly selected sub-samples (10 cm x 30 cm) of blue sticky roller traps per plot, then

extrapolating the total numbers of thrips per trap. The number of thrips was counted on four, randomly selected sticky monitoring traps per plot, and then the total numbers of thrips on monitoring traps was extrapolated according to the numbers of traps. The roller trap counts and sticky monitoring trap counts were added together to give a total trap catch estimate. Both estimates are considered a rough approximation and are not directly comparable as the thrips per plot assessments were ‘snapshots’ while the trap counts were cumulative, and both include thrips that may have flown in from adjacent tunnels. In addition, the majority of the sticky monitoring traps that were placed within the strawberry beds were knocked over during the experiments and became contaminated with dirt, so made little contribute to the trapping. However, the estimates are sufficient to test whether enough thrips were caught on traps to reduce the thrips population.

6.2.6.2. Mass trapping with roller traps, with and without the aggregation pheromone.

Following the pilot experiments above, an experiment was set up to test whether roller traps placed along the polytunnel legs, without any monitoring traps in the strawberry beds, are sufficient to reduce thrips numbers and fruit damage and whether there is additional benefit from the aggregation pheromone. The placement of traps in strawberry beds had proven impractical as the traps were knocked over by tractors, whilst placement of traps in the leg rows between strawberry beds can be effective (see 6.3.4.2). The experiment was carried out in a commercial semi-protected strawberry crop (cv. Camarillo, field 10, Table 2.1). Each polytunnel was 8.5 m wide and contained five strawberry beds (wider than in the experiments above). Blue sticky roller traps were used in addition to the grower’s usual thrips control programme, which included releases of the predatory mite *N. cucumeris* (fortnightly releases from mid-May to mid-August at 25 mites per plant per release) and three insecticide treatments with spinosad (Tracer) on 18 July, 5 August and 28 August 2012. Blue, yellow and white traps are used widely in commercial crops, but blue traps were used as they typically catch more *F. occidentalis* than yellow or white traps and a narrower range of non-target species (Moreno *et al.*, 1984; Sampson *et al.*, 2012). A data logger was placed in a white delta trap to record temperature and humidity (see Chapter 2).

On 9 July 2012, the experiment was laid out in a randomised design with three treatments and three replicate plots (Figure 6.3). Two weeks before the start of the experiment the thrips distribution through the field was surveyed by counting the numbers

of adult thrips per medium-aged flower by eye, using a x7 head lens (optiVISOR), in 10 flowers from 10 different 4 m² plots (n=100) located in a zig-zag pattern across the field. The experiment was then sited in an area of the field that had been shown to have an even distribution of thrips. Each plot was 17.6 m long and 8.5 m (one tunnel) wide, with 33 m between plots within tunnels and 25 m between plots in different tunnels. All plots were located at least 40 m in from the ends of the polytunnels to reduce edge effects.

Treatments were:

- Control plots without any traps or lures;
- Trap plots with blue sticky roller traps (Optiroll, 100 m x 30 cm, cut to plot length, Russell IPM Ltd);
- Pheromone plots with blue sticky roller traps (Optiroll) plus lures containing the *F. occidentalis* aggregation pheromone (Thripline_{ams}).

The blue sticky roller traps were double-sided and placed down both sides of the treatment plots, clipped onto the polytunnel legs between strawberry beds so that they did not interfere with work on the crop. The base of each trap was level with the crop canopy (approximately 30 cm above the ground). The pheromone lures, each containing 30 µg neryl (*S*)-2-methylbutanoate, were pushed into a hole made in the blue sticky roller trap with a hole punch beside every tunnel leg (2.2 m apart as above, 18 lures per plot). An assessment of thrips numbers was made on 9 July 2012 before the traps were put up, then at approximately monthly intervals on 8 August and 10 September. On each assessment date, 40 medium-aged flowers and 20 fully swollen white fruit were sampled regularly from across each plot, excluding 2.2 m in from the ends to reduce edge effects, as above. The numbers of adult thrips per flower and the numbers of seeds surrounded by bronzing per fruit were counted by eye using a x7 head lens (optiVISOR), as above. Flowers were placed in 80% alcohol so that thrips could be extracted and identified to species. These pooled samples were used to calculate the percentage of *F. occidentalis* in the eye counts (section 6.2.8). The numbers of flowers per plant were counted on 10 plants from the middle of the trial area on each assessment date.

On 10 September 2012, the numbers of larval thrips per flower were counted by eye as above, at the same time as the adult counts. Such counts should be interpreted with caution as only the larger larvae will be visible and the counts are not as reliable as counts of adults

(González-Zamora & Garcia-Marí, 2003). However, they would give a relative indication of changes in a population.

To test the impact of trapping on thrips density, simple estimates of the numbers of adult thrips per plot (on each sample date) and thrips per roller trap (at the end of the experiment) were made, as above. Numbers of adult thrips per plot were estimated by multiplying the mean numbers of adult thrips per flower by the mean numbers of flowers per plant and plants per plot (set by the planting density), as above. The numbers of thrips on roller traps were estimated by counting the total numbers of thrips on six randomly selected sub-samples (10 cm x 30 cm) of blue sticky roller traps per plot, then extrapolating the total numbers of thrips per trap.

6.2.6.3. *Thrips identification*

The flower samples collected during the mass trapping experiments in 2012 were rinsed in alcohol to remove the adult thrips and were pooled by date and site. *Frankliniella occidentalis* was separated from other species by eye under a binocular microscope, and then sub-samples of thrips were placed on microscope slides to confirm the identification under a compound microscope (see Chapter 2). Confirmation of the identification (on slides) was carried out on 50 randomly selected specimens considered to be *Frankliniella* spp. and 50 randomly selected specimens considered to be *Thrips* spp. per month for each mass trapping experiment, using the methods detailed in Chapter 2. If fewer than 50 *Frankliniella* spp. (early in the season) or *Thrips* spp. (early and late in the season) were available, then all were identified.

6.2.7. **Cost-benefit analysis of mass trapping with and without pheromone.**

The data from the experiment above (section 6.2.6.2) were used to calculate the cost of trapping and estimated returns to growers. The cost of the different treatments was compared to the estimated returns based on the price of damaged and undamaged fruit. An economic injury level of bronzing around 30 seeds per harvested red fruit, which was about 10% of the fruit surface bronzed, was used to separate higher priced fruit (class 1 fruit) from fruit that had been downgraded to a lower price (class 2 fruit). This was derived from damage recorded on first and second class fruit (*cv.* Camarillo), that had been graded by staff at the commercial packhouse at the time of the trial (see Chapter 4). As fruit bronzing can be caused by environmental factors as well as thrips (Koike *et al.*, 2009),

a regression analysis was used to confirm the relationship between fruit bronzing and thrips density using mean bronzing on white fruit and mean thrips per flower in the nine plots in the mass trapping experiment in September 2012.

The economic injury level from the pack-house was assessed on red fruit whereas the plot data were obtained from white fruit (where damage shows up more easily), so a conversion factor was required to predict the economic injury level on white fruit. To quantify the conversion factor, bronzing was assessed on white fruit in 32 marked plots of 0.5 m² (cv. Camarillo, field 4, Table 2.1) on 5 September 2012, then on red fruit (before picking) in the same plots on 10 September (when the white fruit from 5 September had turned red). On each date, the numbers of seeds surrounded by bronzing was assessed by eye, using a x7 head lens (optiVISOR), on five fruit per plot. The conversion factor was quantified by regressing bronzing on white fruit (from 5 September) on bronzing on red fruit (from 10 September) using a square root transformation to normalise the data.

The economic returns to growers from trapping were calculated by subtracting the total cost of trapping per hectare (including blue sticky roller traps, pheromone lures and the cost of labour to erect the traps once for the period July to September) from the estimated increase in fruit sales per hectare during September (estimated sales in treated plots minus estimated sales in control plots). The weight of fruit sold (class 1 and class 2 combined, in kg per ha) was assumed to be the same for all treatments and was based on the actual yield per ha in the crop for the month of September, although this may have underestimated the weight in treated plots as a slightly higher weight may be associated with lower thrips numbers (Nondillo *et al.*, 2010). This would have underestimated the return on trapping. Earlier months were discounted as there were insufficient thrips to cause fruit downgrading. The economic injury level, derived from harvested red fruit with the white fruit conversion (see above), was used to calculate the proportion of class 1 and class 2 fruit in each plot. Fruit sales were then projected based on the proportion of class 1 and class 2 fruit and sale price of each. Prices for class 1 and class 2 fruit were taken from an average of grower prices from buyers for five supermarkets, for cultivar Camarillo in 2012.

6.2.8. Statistical analysis

Statistical methods are described in Chapter 2 (section 2.8).

6.3. Results

The spectral reflectance of the different trap types used in this chapter is shown in Figure 6.4. One reading on the blue Impact traps with adhesive was anomalously high and was thought to have hit a thicker spot of glue and was omitted from the data. The results are expressed as a percentage of the reflectance from white barium sulphate, which is an efficient reflector of all the wavelengths across the spectrum. The results show that the three blue trap types tested (Impact trap, Takitrap b and Optiroll) all have similar reflectance in the absence of adhesive and all three matched the same shade of light blue by eye (north, indirect daylight), number 299 U, in Pantone colour charts (colour selector 1000, uncoated). The addition of adhesive to the Takitrap b blocked most UV reflectance, yet the Impact trap adhesive increased the UV reflectance around 350-400 nm. No information was available on the type of adhesives used by the different companies and no assessment was made of the impact of the different glue types on trap catch. The white Impact trap shows a standard white reflectance, but UV-absorbing for the most part and with a peak in the blue (around 400-450 nm). This could either be due to an element of fluorescence, indicated by a reflectance over 100%, or due to specular reflectance over a shiny surface.

6.3.1. What is the effect of trap colour on catch of pest and beneficial species?

There was no significant effect of trap colour (blue vs white) on trap catch of *F. occidentalis* ($F_{(1, 13)} = 1.1$, $P = 0.32$) (Table 6.1). There was a strong effect of trap colour on the other pest species assessed: white traps caught significantly more *T. major* ($\times 2$) ($F_{(1, 13)} = 26.9$, $P < 0.001$) and *L. rugulipennis* ($\times 4$) ($F_{(1, 13)} = 12.8$, $P = 0.003$) than blue traps (Table 6.1). One of the traps had been knocked over during the experiment, so the affected block was omitted from the analysis.

Beneficial insects, including bees (the main pollinators of strawberry), predators and parasitoids, were caught in very low numbers on both trap colours. There was a significant effect of trap colour (all species combined) on beneficial insects: white traps caught significantly more beneficial insects ($\times 2$) than blue traps ($F_{(1, 13)} = 5.4$, $P = 0.036$) (Table 6.1).

There was a significant effect of trap colour on non-target species (all species except thrips): white traps caught significantly more insects ($\times 2$) than blue traps ($F_{(1, 13)} = 16.4$,

$P = 0.001$) (Table 6.1). The majority of these insects were dipterans that were not identified to species, but were not considered to be pests as no dipteran pests were recorded by the grower or his advisors during weekly pest monitoring (S. Clarke, pers. comm, 2012).

As both trap colours were equally attractive to *F. occidentalis*, but blue traps were more selective (attracting fewer beneficial species and non-target insects), blue traps were used in the mass trapping experiments (see 6.2.6).

6.3.2. Can thrips escape from sticky traps?

Monitoring traps lost about 14% of the daily trap catch overnight, in a field with few thrips ($F_{(1, 9)} = 6.5$, $P = 0.031$, Figure 6.5), which suggests that thrips were escaping from the traps. Only two thrips were caught on the 10 traps that were only out overnight. There were significantly more thrips ($\times 1.7$) touching the edge of traps that were out over 23 hours (8.0 ± 0.7) compared to traps that were only out during the day (4.8 ± 0.9) ($F_{(1, 9)} = 13.2$, $P = 0.005$), which adds to the evidence that thrips may be moving to the edge of the traps before escaping from the glue (as observed by W. Kirk, pers. comm, 2012). Temperatures were suitable for thrips activity throughout the experiment, with a mean day-time (9.00 h to 17.00 h) temperature of 21 °C (range 19-24 °C) and a mean night-time (17.00 h to 8.00 h) temperature of 16 °C (range 13-22 °C).

6.3.3. Do traps decrease in efficacy through time?

The daily trap catch of thrips on monitoring traps decreased with the length of time that the traps were in place ($F_{(2, 18)} = 90.9$, $P < 0.001$) (Figure 6.6). The highest total trap catch over four days occurred when traps were replaced daily. There was a 25% decrease in total trap catch when traps were replaced every two days and a 30% decrease when traps were left in place for four days, so the decrease in efficiency was greatest soon after the traps had been deployed.

6.3.4. Trap placement in commercial strawberry

6.3.4.1. Does trap height and orientation affect trap catch?

The height above the crop and orientation of traps placed above a strawberry crop had a significant effect on trap catch ($F_{(4, 76)} = 14.6$, $P < 0.001$) (Figure 6.7). Traps caught most thrips when suspended vertically, with the base approximately 10 cm above the crop,

compared to other positions tested. Traps placed horizontally or 20 cm higher above the crop caught significantly fewer thrips (both $\times 0.6$) (Tukey's test, $P < 0.001$). There was no significant difference in the trap catch of vertical traps placed in landscape or portrait orientations at the same height above the crop (Tukey's test, $P = 0.98$). The cardinal direction of the trap did not affect the total trap catch (Tukey's test, $P = 0.07$), although the thrips were not evenly distributed on the traps: On the south-facing traps there were significantly more thrips ($\times 2$) on the shaded (north) side than the brighter (south) side of the traps ($F_{(1, 38)} = 5.1$, $P = 0.03$), yet there was no significant difference in the numbers of thrips on the front or back of west-facing traps ($F_{(1, 38)} = 1.5$, $P = 0.23$). This distribution of thrips on traps (more on the north side of the traps) did not appear to relate to wind speed or direction as it was a relatively still day (windspeed of 7 mph, wind direction ENE), and the same pattern was observed on south-facing traps in the trapping experiments carried out in Spanish pepper and UK strawberry crops (C. Sampson, unpublished data, 2011), where the predominant wind direction differed. On the horizontal traps, there were significantly more thrips ($\times 6$) on the top surface of the traps (a mean of 289 ± 52) than on the bottom surface (a mean of 52 ± 15) ($F_{(1, 39)} = 19.2$, $P < 0.001$), suggesting that the traps may be attracting flying thrips from above the crop rather than thrips from the flowers below (i.e. traps may not be initiating thrips take-off), although the thrips could be flying around the traps to land on the top.

6.3.4.2. Are traps effective when placed between the tunnels?

More thrips were caught on traps placed within strawberry beds than on traps placed in the area between the tunnels where the polytunnel legs reach the ground ($\times 1.2$) ($F_{(1, 17)} = 7.2$, $P = 0.016$), however, there was a significant interaction between thrips sex and trap placement ($F_{(1, 34)} = 60.7$, $P < 0.001$), indicating a different response to trap placement between the sexes. There were significantly more female thrips ($\times 1.3$) ($F_{(1, 17)} = 15.6$, $P < 0.001$), yet significantly fewer male thrips ($\times 0.6$) ($F_{(1, 17)} = 30.2$, $P < 0.001$) on traps placed between the tunnels compared to those placed in strawberry beds (Figure 6.8). The sex ratio of thrips collected from strawberry flowers in the crop was similar to that on traps placed between the tunnels, 44% male and 47% male respectively, but there were proportionally more males (64% male) on the traps placed above the strawberry beds.

As the traps placed between the tunnels, in the area where the tunnel legs reach the ground, caught more female thrips than traps placed above the strawberry beds, the

placement of traps between tunnels could have a greater impact on thrips population development, as well as being more practical for growers, so this placement was used for the mass trapping experiments (see 6.2.6).

6.3.5. Efficiency of trapping through the season

Thrips numbers increased steadily from when the strawberry plants came into flower in mid-May 2011 (Figures 6.9 A, B), then rapidly in the second half of July and remained high for the rest of the season until October, when they declined again. Blue sticky monitoring pheromone traps caught large numbers of thrips, averaging over 800 thrips per monitoring trap per week (mid-May to mid-September) but exceeding 2000 thrips per monitoring trap per week on occasions. Each monitoring trap caught the number of thrips per week equivalent to all the adult thrips in an area of about 9 m² of crop, on average. Trapping efficiency (numbers of thrips per trap / numbers of thrips per m²) was greatest in the brief period between mid-May and early-June, when flower numbers were below 10 per m² and adult thrips numbers were low (Figure 6.9 D). The trapping efficiency dipped from mid-June to late July when the crop was in full flower flush (30-70 flowers per m², Figure 6.9 A) and thrips numbers still low (Figure 6.9 B), but increased to intermediate levels throughout the main cropping period (August-September), when flowers averaged about 20 per m² and adult thrips numbers were >4 per flower (Figure 6.9 D). A spike in trap catch at the end of August occurred during thundery weather (Figure 6.9 C) (Kirk, 2004), and a second spike in trapping occurred at the end of September when a strawberry crop in the adjacent field was being pulled out during warm weather (max 33°C) (Figure 6.9 C). The sharp drop in trapping efficiency at the end of the season corresponded with falling temperatures (from a weekly mean maximum of 31.6°C to 20.1°C) in early October.

6.3.6. Can mass trapping reduce thrips numbers and fruit damage?

6.3.6.1. Pilot experiment in a first-year and a second-year crop

At the start of the experiment in April 2012, low numbers of thrips were found in flower samples from the second-year crop but none were found in samples from the first-year crop (n = 320 flowers). By May, thrips were present in both crops, but in low numbers (<1 per flower), so it was not possible to show a reduction in thrips numbers as a result of mass trapping (Figure 6.10 A, B). Thrips numbers rose rapidly in both crops at

the end of July. Mass trapping with blue sticky roller traps and blue sticky monitoring traps with additional *F. occidentalis* aggregation pheromone and plant volatiles lures, reduced thrips numbers by 67% and 60% in June; by 78% and 51% in July; and by 48% and 60% in August, in first and second-year crops respectively (Figure 6.10 A, B). The results of the two fields were combined for analysis and show that the reductions in thrips numbers from mass trapping were significant in June ($F_{(1, 10)} = 14.0$, $P = 0.004$), July ($F_{(1, 10)} = 7.4$, $P = 0.022$), and August ($F_{(1, 10)} = 9.0$, $P = 0.013$) (Table 6.2). There was no significant difference in thrips numbers between treated and untreated plots in April (before the start of the experiment) ($F_{(1, 10)} = 0.8$, $P = 0.40$), or May ($F_{(1, 10)} = 0.6$, $P = 0.46$). Analysis of effects showed no interaction between the treatment effect and the field (first-year or second-year crop), indicating that the response to treatment was the same in both crops (e.g. Treatment*field interaction in August, $F_{(1, 9)} = 0.05$, $P = 0.82$).

To get a relative measure of the impact of trapping on the thrips population, trap catch (cumulative total) in June and August, and thrips density (on each assessment date) were compared (Table 6.2). Although the estimates are not directly comparable as the thrips per plot assessments were ‘snapshots’ while the trap counts were cumulative, the estimates are sufficient to confirm that trapping would have a considerable impact on the population. On each occasion, more thrips had accumulated on the traps over the two months that they were up, than were present in the control plots without traps by factors of $\times 1.9$, $\times 1.3$ (June and August, 1st year crop), $\times 1.4$ and $\times 1.4$ (June and August, 2nd year crop) (Table 6.2).

In the first-year crop it was not possible to show a reduction in fruit damage in June from mass trapping, as thrips numbers were very low in the control, but there was a significant reduction in fruit damage by 67% and 29% in July and August respectively (Figure 6.10 C). Mass trapping in the second-year crop reduced fruit damage by 54% and 49% in June and July respectively (Figure 6.10 D). Some of the fruit damage recorded on fruit in June, in the second-year crop, appeared to be the result of pesticide scorching as the bronzing was evenly distributed over the exposed areas of the fruit, although additional thrips damage was observed. A 52% reduction in thrips damage from mass trapping was recorded in August, but this was not statistically significant as thrips numbers were too low (<1 per flower) to cause significant damage when the fruit were being formed.

Roller traps remained in place for the duration of each experiment and the method of attaching them to the legs was practical and easy to apply. Although the traps are designed

for outdoor use with non-drying glue, placement in the leg area meant that they were subject to water dripping (or pouring) from the tunnel cladding in places, which washed off thrips and glue in some areas. 2012 was an exceptionally wet season with approximately 457 mm rainfall from April to July in the West Midlands, which was 190% average rainfall (1981-2010) (<http://www.metoffice.gov.uk>, accessed 2 Oct 2013). Thrips were seen walking over the traps in areas where the glue had been washed away. Traps placed within strawberry beds on bamboo canes were vulnerable to tractor damage. After one month, 29 out of 72 traps per field within beds were still in place (40%). After two months an average 5 out of 72 traps were still in place (5%). Those traps that remained were soiled, broken and often no longer sticky. So few thrips were recovered from these traps (< 5 per trap) that they were discounted from Table 6.2 as the counts were not reliable, resulting in an underestimate of the numbers of thrips caught by trapping.

Maximum daytime temperatures were generally cool before the end of May, although they exceeded 20°C for short periods on most days (Appendix B), which is needed for *F. occidentalis* flight and trapping (O'Leary, 2005).

6.3.6.2. Mass trapping using roller traps with or without aggregation pheromone

Thrips were well controlled until mid-August. As thrips numbers in the plots without traps were low in early-August (<1 per flower), it was not possible to show a reduction in thrips numbers ($F_{(2,6)} = 1.55$, $P = 0.29$) or fruit damage ($F_{(2,6)} = 3.29$, $P = 0.11$) with trapping at this time (Figure 6.8). The thrips population took-off in August when the population was largely *F. occidentalis* (Figure 3.4 D), which was not well controlled by spinosad (Tracer). Mass trapping with blue sticky roller traps alone, or with additional *F. occidentalis* aggregation pheromone, reduced thrips numbers by 61% and 73% ($F_{(2,6)} = 60.1$, $P < 0.001$) and fruit damage by 55% and 68% ($F_{(2,6)} = 13.29$, $P = 0.006$) respectively by early September (Figure 6.11). Trapping relies on thrips flight and maximum day-time temperatures exceeded 20°C on all but two days during the experiment, which was sufficient for *F. occidentalis* flight (O'Leary, 2005).

Counts of thrips larvae by eye on 10 September showed that mass trapping with blue sticky roller traps alone, or with additional *F. occidentalis* aggregation pheromone, reduced larval thrips numbers by 63% and 90% respectively ($F_{(2,6)} = 14.1$, $P = 0.005$). The mean number of larvae per flower (\pm SEM) was 1.83 ± 0.49 on control plots without traps, 0.68 ± 0.15 on plots with traps alone and 0.19 ± 0.07 on plots with traps and pheromones.

Tukey's test showed that plots with traps alone and plots with traps with pheromone had significantly fewer larvae than the control plots ($P = 0.044$ and $P = 0.005$ respectively), but that the difference between the two treatments with traps was not significant ($P = 0.17$).

To get a relative measure of the impact of trapping on the thrips population, trap catch (cumulative total) in September and thrips density (on each assessment date) were compared. The traps were estimated to have caught $13,376 \pm 476$ (without pheromone) and $25,754 \pm 1,844$ (with pheromone) thrips per plot. Thus, the addition of the *F. occidentalis* aggregation pheromone to blue sticky roller traps approximately doubled the trap catch ($F_{(1,4)} = 64.2$, $P < 0.001$). The numbers of thrips on plants were estimated as 924 ± 137 (July), $2,228 \pm 566$ (August) and $13,255 \pm 832$ (September) thrips per plot in plots without traps; $1,166 \pm 308$ (July), $1,387 \pm 402$ (August) and $5,188 \pm 734$ (September) thrips per plot in trap plots; and 704 ± 230 (July), $1,341 \pm 120$ (August) and $3,630 \pm 188$ (September) thrips per plot in pheromone trap plots. Pheromone traps had accumulated nearly 26,000 thrips per plot over three months, at a time when numbers of thrips per plot were about 13,000 in the plots without traps. The numbers confirm that trapping would have a considerable impact on the population.

6.3.7. Cost-benefit analysis of mass trapping with and without pheromone.

The percentage of class 1 and class 2 fruit, the projected fruit sales in treated and untreated plots and the return on trapping investment are shown in Table 6.3.

The cost of treating every tunnel with blue sticky roller traps was based on 13 traps x 100 m at £25 per trap, plus labour costs based on three workers taking 1.5 days per hectare (£252 per ha at £7 per hour wages) and the cost of pheromone monitoring lures at £2.34 per lure (Table 6.3). Different (possibly lower) prices are likely to apply if the pheromone was formulated for mass trapping and registered as a control method. Fruit sales were calculated based on £2.99 per kg for class 1 fruit and £1.21 per kg for class 2 fruit and a total yield of 6,262 kg per ha during September (for all treatments).

The amount of bronzing on class 1 and class 2 fruit used an economic injury level of 10% of the red fruit surface bronzed (see Chapter 4), which was around 30 seeds on red fruit. A regression of white fruit bronzing on red fruit bronzing was significant ($F_{(1, 30)} = 56.3$, $P < 0.001$; $R^2 = 64\%$) ($y = 1.10 + 0.97 x$; where y = square root of white fruit bronzing and x = square root of red fruit bronzing). Using this equation, a threshold of

bronzing of 30 seeds on red fruit was equivalent to bronzing of around 41 seeds on white fruit. The amount of bronzing was rather similar between fruit stages in this experiment and so the accuracy of conversion of white fruit damage scores to red fruit damage scores made little difference to the cost-benefit analysis. A regression of mean bronzing on white fruit on the mean numbers of thrips per flower in the different plots in September 2012 was consistent with bronzing being caused by thrips ($F_{(1,7)} = 36.4$, $P < 0.001$, $R^2 = 82\%$) ($y = 0.98 + 0.84 x$; where $y = \log$ white fruit bronzing and $x = \log$ adult thrips per flower).

The cost-benefit analysis demonstrates a return on investment for mass trapping for both blue sticky roller traps and blue sticky roller pheromone traps. This assumes that the mass trapping is used in addition to other methods such as predatory mites, as part of an integrated pest management programme. If the return on investment of £2,200 per ha (Table 6.3) is typical, then mass trapping could save UK strawberry growers over £2 million per annum, projected over the 1000 ha of everbearer strawberry varieties that are susceptible to *F. occidentalis* damage (R. Harnden, pers. comm., 2013).

6.3.8. Thrips identification

The proportion of *F. occidentalis* in strawberry flowers increased through the season from below 60% before July to over 95% by the end of August in 2011 and 2012 (see Chapter 3, Figure 3.4). Other thripid species found as adults included *T. major*, *T. tabaci*, *Thrips fuscipennis*, *F. intonsa*, *Frankliniella tenuicornis*, *Thrips angusticeps* and *Thrips atratus*.

The proportion of *F. occidentalis* was high in the strawberry fields where the sticky monitoring trap experiments were carried out during August (2011 and 2012): experiment 6.3.1, 83% *F. occidentalis* ($n = 190$); experiments 6.3.2, 6.3.3 and 6.3.4.2, 97% *F. occidentalis* ($n = 72$); experiment 6.3.4.1, 92% *F. occidentalis* ($n = 96$).

6.4. Discussion

This study confirms that mass trapping can reduce thrips numbers, as found previously in strawberry (Lim & Mainali, 2009) and pepper (Lim *et al.*, 2013). However it goes further than previous work by demonstrating that the reduction in thrips numbers reduces crop damage and that it can also increase grower economic returns. Further work is

needed to optimise the timing of trapping, to make the best use of the aggregation pheromone and to determine the best trap colour, adhesives and scents to use in different cropping systems.

The best trap colour for mass trapping may vary according to the importance of secondary pests and beneficial insects in the crop and the impact of trapping on these non-target species. Blue and white sticky traps caught similar high numbers of *F. occidentalis* in the trap colour experiment (Table 6.1), which was an unexpected result given that *F. occidentalis* are most attracted to light blue traps (Brødsgaard, 1989). Careful examination of the colour spectrum of the blue and white traps used offers some explanation (Figure 6.4). The trap catch on the blue traps used may have been slightly lower than expected as the colour (299 U in Pantone colour charts, colour selector 1000, uncoated) was close to, but not an exact match to the most attractive trap colour identified by Brødsgaard (1989) (257 in Pantone colour charts) and the adhesive on the traps increased UV reflectance (Figure 6.3), which is known to reduce trap catch (Vernon & Gillespie, 1990). The trap catch on the white trap tested may have been slightly higher than expected as they had low UV reflectance and a distinct fluorescence in the blue wavelengths (around 400-450 nm) (Figure 6.3) giving the white traps a very bright appearance, and thrips may have seen the white traps as 'blue'. The results suggest that further improvement in trap catch would be possible by careful selection of trap colour and adhesive. The type of adhesive used can alter the spectral properties of the traps, so plays an important part in trapping that is rarely mentioned in the scientific literature.

As expected (Kirk, 1984), the white traps with low UV were less selective than the blue traps tested and caught about twice as many non-target insects (Table 6.1). This could be an advantage in crops where more than one economically-damaging pest is trapped. Two species of secondary pest were caught frequently on traps in this study: *Thrips* spp. can cause fruit damage when present in sufficient numbers (de Kogel, pers. comm, 2011) and *Lygus* spp. cause fruit deformation when present in very low numbers (Easterbrook, 2000), so mass trapping with white traps could be advantageous if they reduce *Lygus* spp. populations. One disadvantage is that contamination of the traps with non-target species reduces the efficiency of the traps against *F. occidentalis*, which was the most damaging pest species present. Further experiments would be useful to test the long-term efficiency of blue and white traps and to determine their impact on secondary pests.

Relatively few beneficial insects were caught on blue or white sticky traps (<5 per trap) compared to *F. occidentalis* (>200 per trap) (Table 6.1), but no assessment of the field populations of beneficial insects was made, so the impact of trapping on natural enemies could not be determined. In the fields used, predatory mites were released for thrips and spider mite control but no flying natural enemies had been released and few were observed in the flower samples, so it is likely that the low trap catch reflected low numbers in the crop. *Bombus terrestris* (Natupol, Koppert, Rodenrijs, NL) were used for pollination in all the experimental fields, so were present throughout the crops visiting all the flowers, yet few bumble bees were caught on traps of either colour. The increase in percentage class 1 fruit in the mass trapping experiments (Table 6.3) indicates that the blue roller traps did not disrupt pollination, as there would have been more downgraded misshapen fruit if pollination had been affected. Further work is required to determine the impact of mass trapping on beneficial insects, but blue traps are likely to be the better choice for mass trapping where *F. occidentalis* is the main pest problem as there would be less contamination of traps by non-target species and fewer natural enemies caught.

The adhesive on sticky traps is a key part in efficiency and this study showed that a significant number of thrips (14% per day) may be escaping from the sticky traps (Figure 6.5), as found by Chu *et al.* (2006). An alternative explanation for the decline in thrips numbers overnight is that they are being predated, but there was no evidence of this as there were few predators caught on the traps. The escape from traps corresponded with an increase in thrips numbers touching the edge of the traps, supporting the observation (Kirk, pers. comm., 2011) that the thrips move to the edge and use it to escape the glue on the trap. However, although there is no data on the viability of escaped thrips and it seems unlikely that glue covered thrips would thrive. It was observed that the traps are less tacky in cool conditions, so there may be differential escape at different temperatures, but this was not tested. It would also be useful to know whether there is an increased escape rate from traps with additional aggregation pheromone, because this activates thrips. Escape from traps explains why trap catch can be improved by adding insecticide to traps (Chu *et al.*, 2006), although this would be less useful against pesticide-resistant *F. occidentalis*. Thicker glue also increases trap catch (Sampson, unpublished data, 2011), which may be the result of reduced escape rate or possibly increased attraction, if the glue changes the spectral properties of the trap. There was a rapid decline in efficiency of the monitoring traps, which became less efficient even after a day in the crop and there was a direct

relationship between trap catch and frequency of replacement (Figure 6.6). Further experiments are required to test whether the efficiency of roller traps, which have a wet glue designed for longer use, decline at a similar rate to that of monitoring traps. Comparison of the cost of traps (including labour) and return on investment (Table 6.3) suggests that the roller traps could be replaced up to three times in the strawberry season (about every 6 weeks) without incurring a loss, but there would be no immediate financial return from replacing traps in crops with little thrips damage.

The trap placement experiments confirm that vertical placement of traps, just above the crop canopy catches the most thrips, as found previously by Gillespie and Vernon (1990) in cucumber and by Laudonia and Viggiani (1998) in strawberry. The cardinal direction of the traps made no difference to the total trap catch, as found by Hoddle *et al.* (2002). An understanding of why more thrips are landing on the north side of traps (regardless of the wind direction) may add further insight into the flight behaviour of thrips. It is possible that the thrips to the north of the traps are more attracted towards traps than those to the south because the traps have a greater contrast with the background, or are lit up by the sun shining through them when viewed from the north with the sun in the background. An alternative explanation, which seems unlikely given that thrips aggregate on visible bright surfaces (Terry & Gardner, 1990), is that thrips are flying around the traps to land on the shaded side. The mass trapping experiments demonstrated that traps within strawberry beds are vulnerable to damage from crop work (section 6.3.6.1) and that placing roller traps along the polytunnel legs between the tunnels is a viable alternative (section 6.3.4.2). More female thrips were caught on traps that were placed between the tunnels in the area where the polytunnel legs reach the ground and if this is more generally true, it is an interesting behavioural result that is unexplained. At the time of the experiment there was a combination of high thrips numbers and low numbers of flowers, so it is likely that thrips were searching for pollen to feed on. It could be that females were more actively searching for new food sources and egg-laying sites away from the (overcrowded) crop than males, as females have a greater need for pollen for egg-production and a greater dispersal response to senescence than males (Rhainds & Shipp, 2003). In contrast, more males were found on the traps in the strawberry beds, which had a higher sex ratio (64% male compared to 44-47% male in flowers or on leg traps), which may relate to swarming behaviour. Males form mating swarms on attractively coloured surfaces and have a greater response to colour than females when swarming (Matteson & Terry, 1992), but it is

unknown whether the aggregation sites are chosen to be close to possible food sources (as would be the case in the strawberry beds). Further studies are required to better understand the dispersal and flight behaviour of male and female thrips. At least on some occasions, placing traps between the strawberry beds, where the polytunnel legs reach the ground, may be more effective for mass trapping than placing them above strawberry beds because the traps catch more female thrips, therefore having a greater effect on the population.

Field monitoring gave an indication that trapping could be effective throughout the growing season, as blue sticky monitoring pheromone traps caught many thrips in every week that the polytunnel covers were in position (mid-May to late-October) (Figure 6.6 C). The efficiency of trapping was similar throughout the season, but possibly better at the start before the first flower flush (Figure 6.9 D). *Frankliniella occidentalis* flight is likely to increase when flowers are scarce because they feed on pollen (Whittaker & Kirk, 2004) and would be searching for food. In addition, starved thrips are known to fly more and have a greater response to colour and odour than satiated thrips (Davidson *et al.*, 2006). This could explain the increased trapping efficiency before the crop had come into full flower. The number of thrips per flower could also play a part by increasing dispersal at high density (Crespi & Taylor, 1990). The broad increases in trap catch and trapping efficiency when maximum temperatures were around 30°C and reduction when maximum temperatures were around 20°C (October) is in line with published information on *F. occidentalis* flight, which showed no take-off at 15°C and increasing flight activity between 20-30°C in a UK population (O'Leary, 2005). Trapping efficiency is likely to decline with the length of time that traps are up, as traps become contaminated by dirt and insects and because the glue loses its stickiness in places. In the mass trapping experiments, thrips could be seen walking over some areas of the blue sticky roller traps after two months in the field. Mass trapping reduced thrips numbers and fruit bronzing when it was at most risk of thrips damage (July to September), but the trapping efficiency index suggests that there might be a benefit in mass trapping from the start of the season (from April), as soon as the polytunnels are erected. Early trapping may keep thrips at a level that can be maintained by the predatory mite *N. cucumeris*, however the returns would not be as high early in the season if thrips numbers remain below the economic injury level through May and June. It would be interesting to test whether mass trapping could be used to reduce the overwintering thrips population. Although temperatures would generally be below optimum for thrips flight (about 20°C), traps could be effective on

warm days as there would be less competition from flowers. Further work is needed to test the impact of using traps at different times of the year and to determine whether, or how often, the traps should be replaced during the season to maximise returns.

The addition of the *F. occidentalis* aggregation pheromone to blue sticky roller traps approximately doubled the trap catch in semi-protected strawberry. This is the first record of using the aggregation pheromone in strawberry (Sampson & Kirk, 2013) and the increase in trap catch is consistent with that found in protected pepper (Hamilton *et al.*, 2005; Sampson *et al.*, 2012), cucumber (Covaci *et al.*, 2012), tomato (Gómez *et al.*, 2006), and top fruit (Broughton & Harrison, 2012), where addition of the pheromone resulted in trap catch increases between 20% and 300%. Increases in *F. occidentalis* trap catch have also been found using plant volatiles and their analogues (Brødsgaard, 1990; Frey *et al.*, 1994; Teulon *et al.*, 1999; Davidson *et al.*, 2007), which attract other thrips species as well as *F. occidentalis*, so they may be useful for mass trapping in crops where there is a complex of thrips pests. The aggregation pheromone has advantages over plant volatiles where *F. occidentalis* is the main thrips pest species as very small quantities are required to elicit a response (Dublon *et al.*, 2008) (<0.5 g per ha was used in this study) and it increases *F. occidentalis* trap catch without directly affecting key natural enemies (Broughton & Harrison, 2012; Sampson *et al.*, 2012). Although a mix of thrips species was present at the beginning of the season in strawberry (see Chapter 3), fruit damage occurred when the thrips population was predominantly *F. occidentalis* (August and September), which is when the aggregation pheromone is likely to be most effective.

As well as reducing the adult thrips population, trapping reduced the larval thrips population by a broadly similar amount, taking into consideration the greater variability of larval counts. Although both adults and larvae can cause damage to strawberries, larvae are the most damaging (Chapter 4) and the results suggest that the reduction in adult population led to fewer larvae, which then led to less damage.

The economic injury level defined from damage assessed on pack-house fruit (see Chapter 4) equated to a density of around six adult thrips per flower in the mass trapping experiment (6.3.6.2), which was within the range of published damage thresholds in strawberry (Steiner & Goodwin, 2005a; Coll *et al.*, 2007a). The economic returns calculated using the economic injury level (Table 6.3) are considered conservative as they do not include loss of fruit that occurred in late August and October, nor do they consider

possible consequential benefits such as reduced spraying costs or reduced numbers of thrips in the following season. In this study a high density of pheromone monitoring lures (every 2.2 m) was used, but if the lures could be spaced at wider intervals without losing efficacy, then the economic returns on trapping would be greater than with traps alone. Further work is required on the range of pheromone attraction in thrips to optimise the spacing and formulation of traps and lures for mass trapping.

This study shows that mass trapping can be cost-effective against a polyphagous, high-density pest species with a short generation time, and integrates well with a pest management programme for *F. occidentalis* in semi-protected strawberry. Mass trapping is unlikely to be sufficient to control *F. occidentalis* on its own and needs to be used alongside other measures. There is plenty of scope for improving the mass trapping by optimising the trap colour and scent, testing different trap sizes and automating the application to reduce labour costs. The cost-benefit calculations, while done in the UK, are likely to be applicable to other countries and other high-value crops such as cucumber and cut flowers.

Table 6.1. The mean number \pm SEM of selected pest species: *F. occidentalis*, *T. major* and *L. rugulipennis*, beneficial species: bees, hoverflies and *Aeolothrips intermedius*, and total non-target species caught on blue or white sticky traps over 96 h in a semi-protected strawberry crop (n = 14). The table shows untransformed means whereas the statistical analysis is on log transformed data.

| Insect type | Mean number \pm SEM of insects caught per sticky monitoring trap (n = 14) | | | |
|--|---|------------------|--------------|--------|
| | Blue traps | White traps | $F_{(1,13)}$ | P |
| Pest species | | | | |
| <i>Frankliniella occidentalis</i> ¹ | 208.8 \pm 17.6 | 234.4 \pm 20.0 | 1.1 | 0.32 |
| <i>Thrips major</i> ¹ | 50.4 \pm 5.6 | 96.0 \pm 8.8 | 26.9 | <0.001 |
| <i>Lygus rugulipennis</i> | 2.8 \pm 1.0 | 11.0 \pm 3.1 | 12.8 | 0.003 |
| Beneficial insects | | | | |
| Bees ² | 0.7 \pm 0.2 | 1.1 \pm 0.4 | | |
| Syrphidae | 0.6 \pm 0.2 | 1.3 \pm 0.5 | | |
| <i>Aeolothrips intermedius</i> | 0.3 \pm 0.2 | 0.5 \pm 0.2 | | |
| Total beneficial insects ³ | 1.8 \pm 0.4 | 4.4 \pm 1.0 | 5.4 | 0.036 |
| All insects (except thrips) ⁴ | 32.1 \pm 3.3 | 66.4 \pm 13.6 | 16.4 | 0.001 |

¹ *F. occidentalis* and *T. major* estimates were extrapolated from sections of trap counts.

² Bees includes solitary bees, bumble bees and honey bees.

³ Total beneficial insects includes coccinellids, predatory mirid bugs (*Anthocoris nemorum* and *Orius* spp.) and parasitic Hymenoptera in addition to those listed above.

⁴ All insects represents whole trap counts of all insects *except* thrips. The majority of these were dipterans that were not considered to be pest or beneficial species.

Table 6.2. Estimated numbers of thrips in flowers per plot and on traps in semi-protected strawberry (cv. Camarillo) and comparison between thrips numbers in untreated plots and plots treated with roller traps, pheromone lures and plant volatile lures, in a 1st and a 2nd year crop. The table shows untransformed means whereas the statistical analysis is on log transformed data.

| Month: | April | May | June | July | August |
|--|-------|-------|-------|-------|--------|
| <i>1st year crop</i> | | | | | |
| Thrips per control plot ^a | 0 | 74 | 428 | 2,370 | 24,443 |
| Thrips per treated plot ^a | 0 | 37 | 142 | 527 | 12,813 |
| Thrips per trap ^b | | | 813 | | 30,646 |
| <i>2nd year crop</i> | | | | | |
| Thrips per control plot ^a | 26 | 2,383 | 1,526 | 1,182 | 10,666 |
| Thrips per treated plot ^a | 16 | 2,107 | 612 | 584 | 4,224 |
| Thrips per trap ^b | | | 2,189 | | 14,850 |
| <i>Analysis of treatment effects^c</i> | | | | | |
| $F_{(1,10)}$ | 0.8 | 0.6 | 14.0 | 7.4 | 9.0 |
| P | 0.40 | 0.46 | 0.004 | 0.022 | 0.013 |

Key:

^a Estimated thrips per plot = Thrips per flower × flowers per plant × plants per plot.

^b Estimated thrips per trap = Thrips counts on trap sections × trap length.

^c Analysis of treatment effect on thrips per plot using analysis of variance in both fields together.

Table 6.3. Cost-benefit analysis of mass trapping in semi-protected strawberry. Comparison of use of no traps with use of blue sticky roller traps, with and without the *Frankliniella occidentalis* aggregation pheromone in a UK crop.

| | No traps (control) | Traps only | Traps with pheromone |
|--|-----------------------|--------------|-------------------------|
| Total cost of trapping (£ ha ⁻¹) | - | 577 | 1,953* |
| Sales of class 1 fruit (£ ha ⁻¹) (%) | 12,482 (67%) | 17,162 (92%) | 18,098 (97%) |
| Sales of class 2 fruit (£ ha ⁻¹) (%) | 2,525 (33%) | 631 (8%) | 252 (3%) |
| Total sales in September (£ ha ⁻¹) | 15,007 | 17,793 | 18,351 |
| Return on trapping (£ ha ⁻¹) | - | 2,209 | 1,391* |

*Cost and return were calculated using current prices of pheromone monitoring lures. If the pheromone were registered and formulated as a control for mass trapping, different prices would apply.

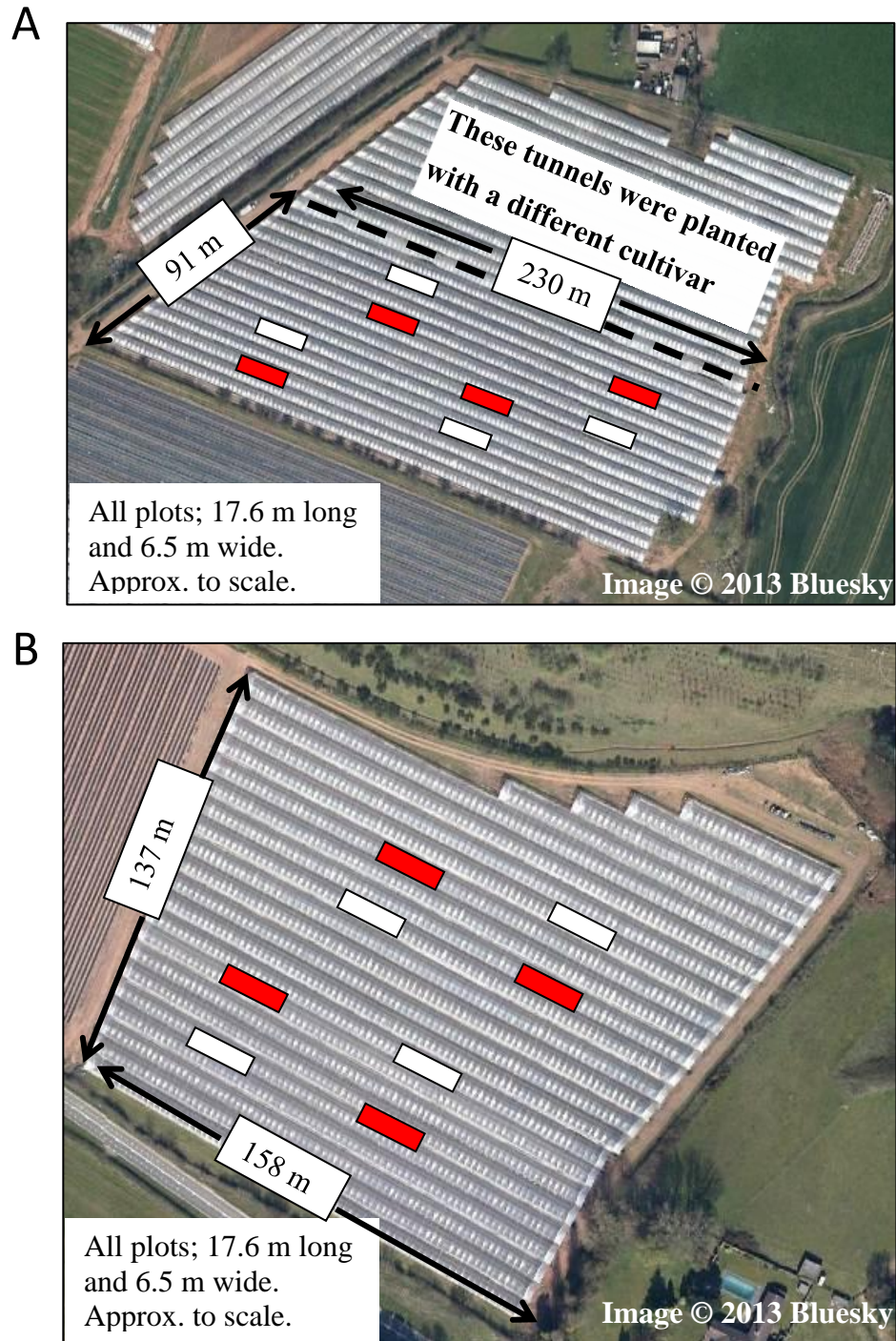


Figure 6.1. Trial layout for the pilot mass trapping experiments in semi-protected strawberry crops (cv. Camarillo), from April to August 2012, showing the approximate location of the control plots in white (without traps or lures) and treated plots in red (with blue sticky roller traps, blue sticky monitoring traps, lures with the aggregation pheromone, neryl (*S*)-2-methyl butanoate, and lures with plant volatile lures) for (A) a first-year crop and (B) a second-year crop in the West Midlands, UK. The images of the greenhouses are from Google Earth (<https://www.google.com/maps>).

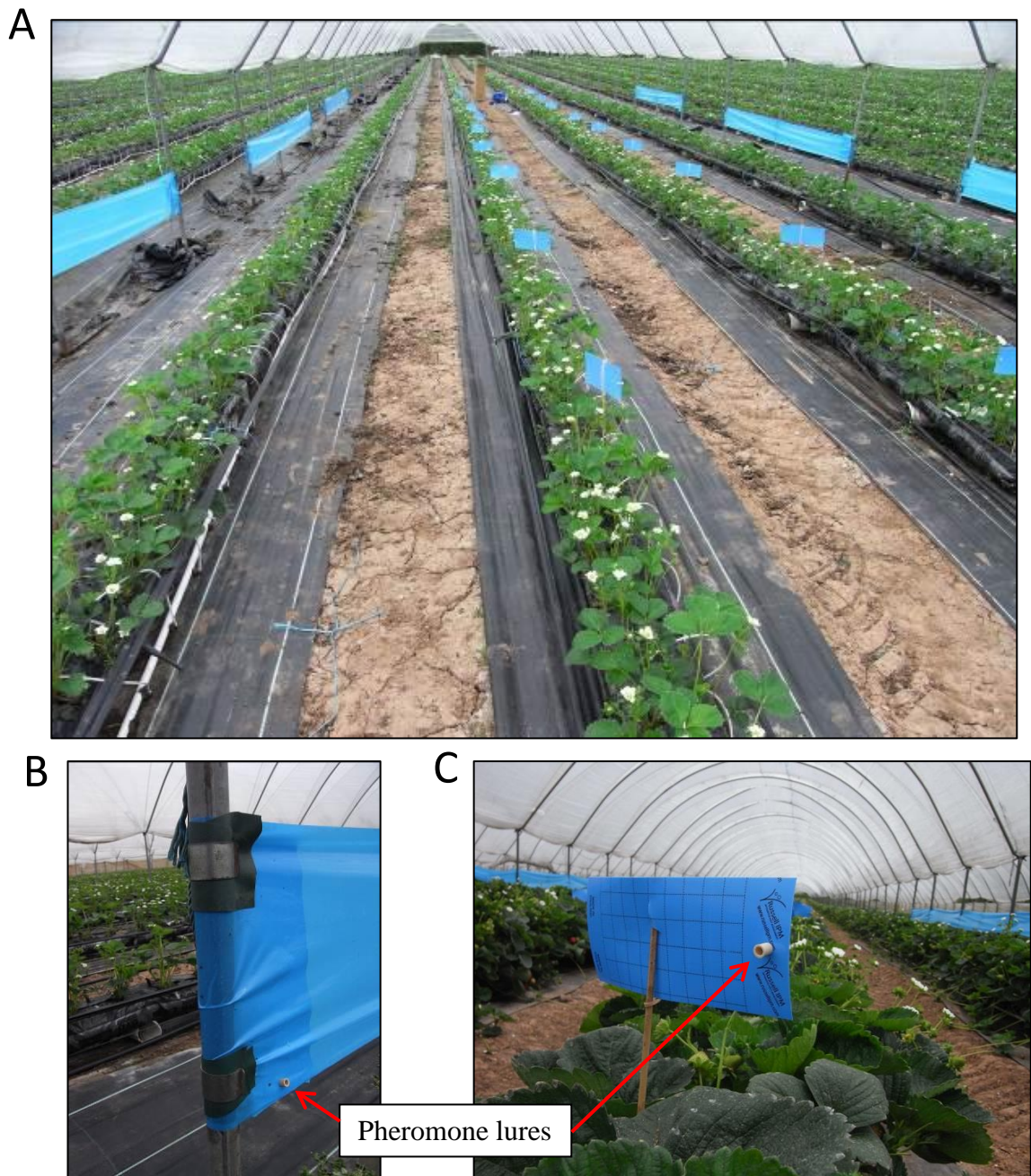


Figure 6.2. A plot from the pilot mass trapping experiments in a first-year semi-protected strawberry crop (cv. Camarillo) showing (A) the initial layout for the treated plots with sections of blue sticky roller trap (Optiroll, Russell IPM) run along the legs of the polytunnels and blue sticky monitoring traps placed on bamboo canes within the strawberry beds (B) the method of securing the roller traps to the polytunnel legs and (C) a sticky monitoring trap secured on a bamboo cane with an aggregation pheromone lure through the string hole.

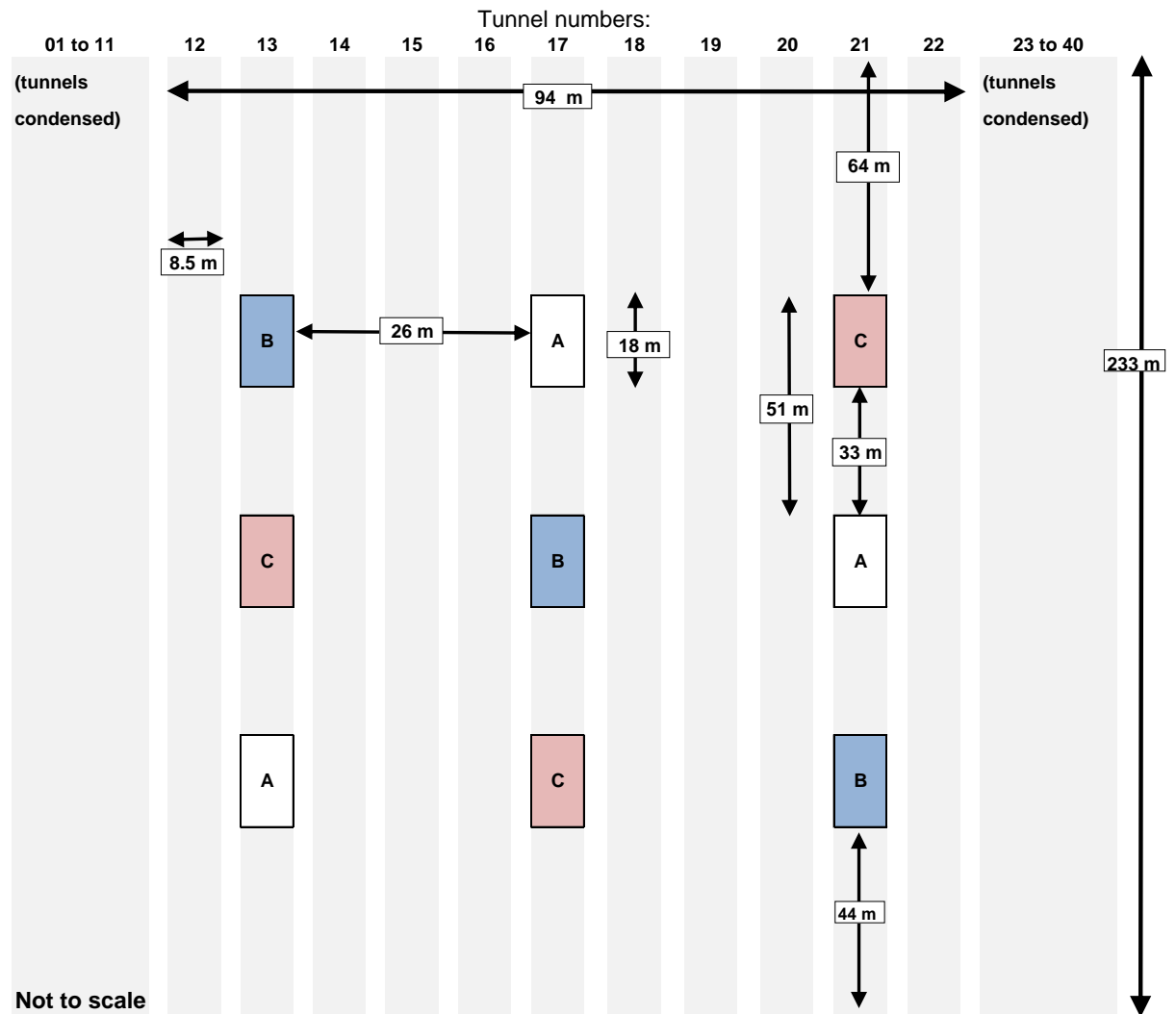


Figure 6.3. Trial layout for the 2012 mass trapping experiment in a semi-protected strawberry crop (cv. Camarillo) in the West Midlands, UK, showing the approximate location of (A) control plots (without traps or lures), (B) trap plots (with blue sticky roller traps (Optiroll, Russell IPM) and (C) pheromone trap plots (with blue sticky roller traps (Optiroll, Russell IPM) and aggregation pheromone lures (Thripline_{ams}, Syngenta Bioline)).

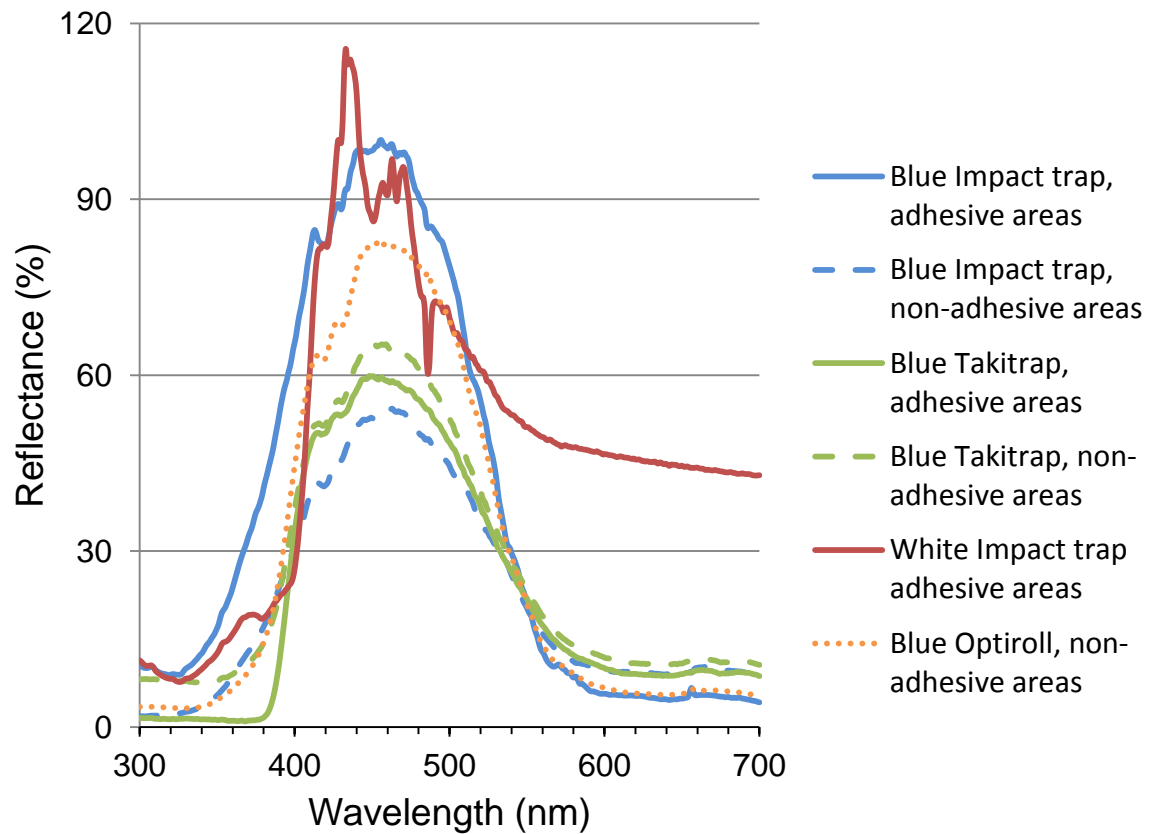


Figure 6.4. The percentage of reflectance in wavelengths from 300-700 nm of the sticky traps types used in this chapter from white barium sulphate. Measurements show the mean of two or three readings and, where available, on areas of the trap with or without adhesive.

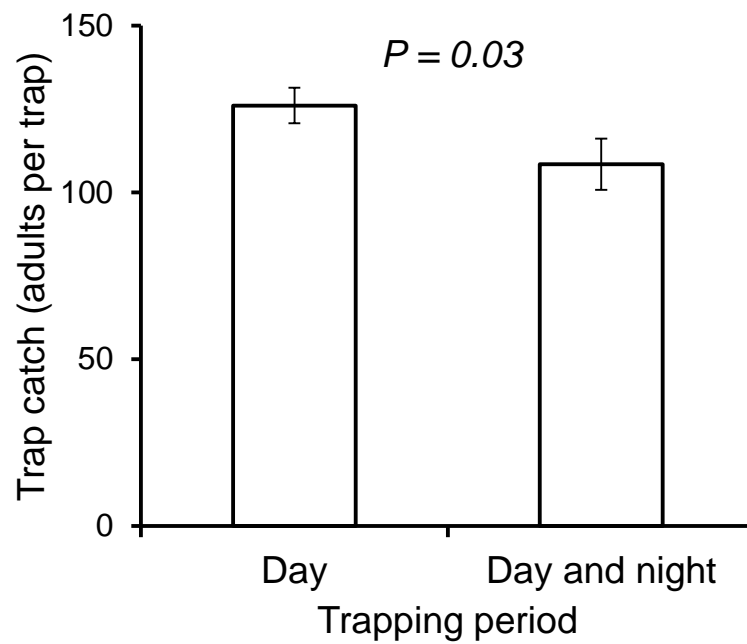


Figure 6.5. The mean trap catch \pm SEM of adult thrips caught on blue sticky monitoring traps comparing day with day and night trap catch ($F_{(1,9)} = 6.5$, $P = 0.031$), in a semi-protected strawberry crop ($n = 10$ traps). The figure shows untransformed means whereas the statistical analysis is on log transformed data.

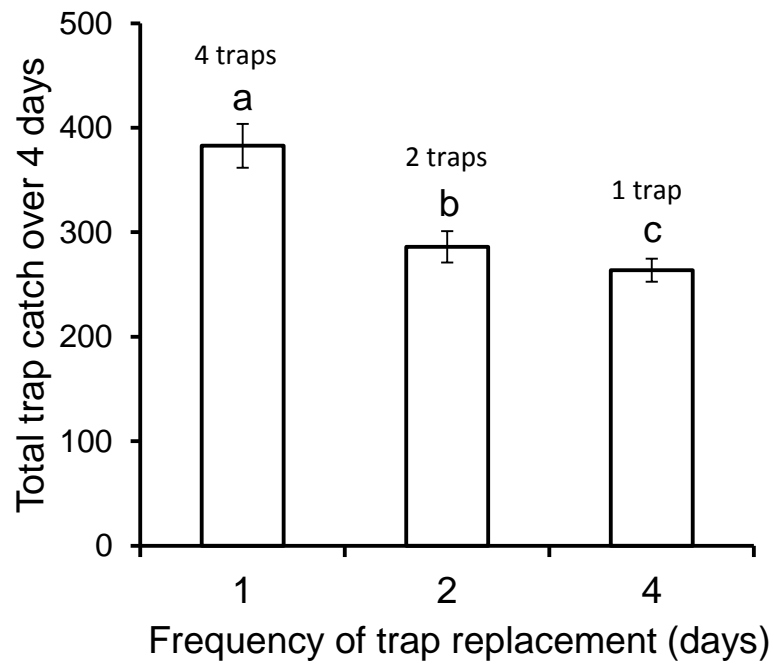


Figure 6.6. The mean trap catch \pm SEM of adult thrips caught on blue sticky monitoring traps over 4 days with traps replaced daily, at two days or not replaced ($F_{(2, 18)} = 90.9$, $P < 0.001$), in a semi-protected strawberry crop ($n = 10$ traps). Means with the same letter are not significantly different (Tukey's test, $P > 0.05$). The figure shows untransformed means whereas the statistical analysis is on log transformed data.

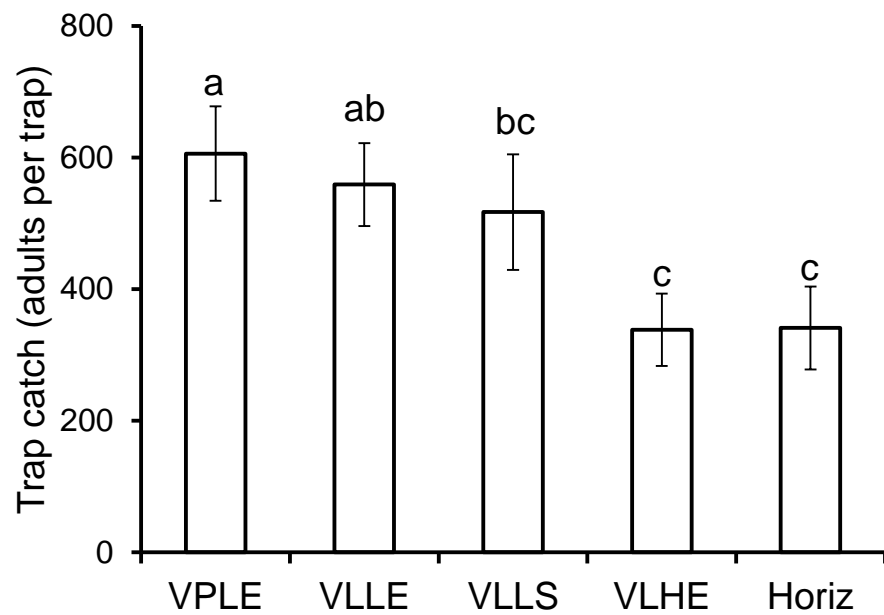


Figure 6.7. Mean trap catch \pm S.E. of thripids on blue sticky monitoring traps in different orientations ($F_{(4, 76)} = 14.6$, $P < 0.001$), above a semi-protected strawberry crop ($n = 20$ traps). Bars with the same letter are not significantly different (Tukey's test, $P > 0.05$). Analysis was on transformed data whilst the chart shows untransformed data.

Key: VPLE = vertical, portrait, low, east facing;
VLLE = vertical, landscape, low, east facing;
VLLS = vertical, landscape, low, south facing;
VLHE = vertical, landscape, high, east facing;
Horiz = horizontal, low.

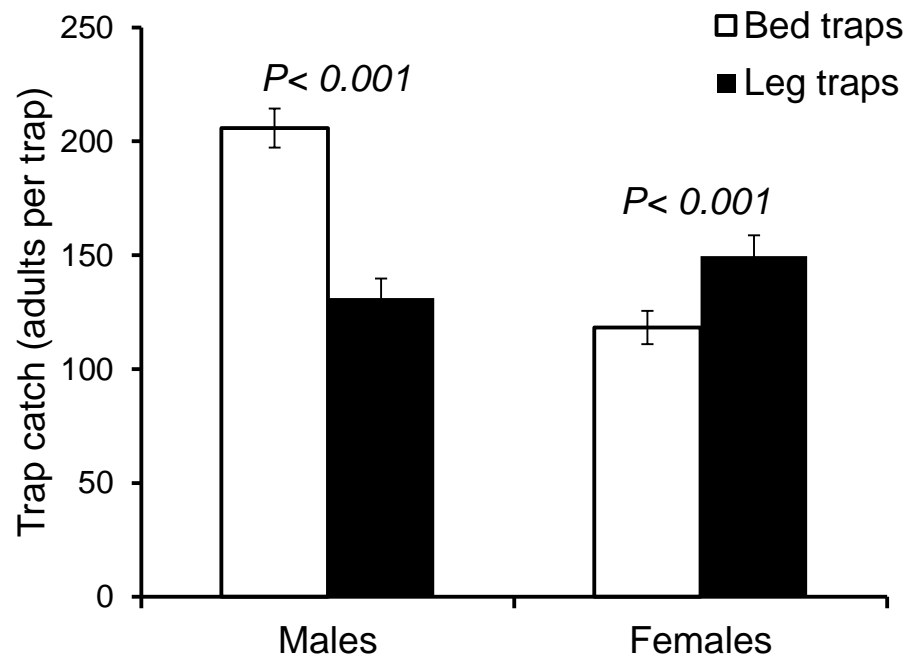


Figure 6.8. Mean trap catch \pm S.E. of thripids on blue sticky traps placed within strawberry beds or in the area between beds where the polytunnel legs reach the ground, over four days in a semi-protected strawberry crop ($n = 18$). Pair-wise comparisons between trap catch of male and female thripids on traps in and between strawberry beds are shown by P values. Analysis was on transformed data whilst the chart shows untransformed data.

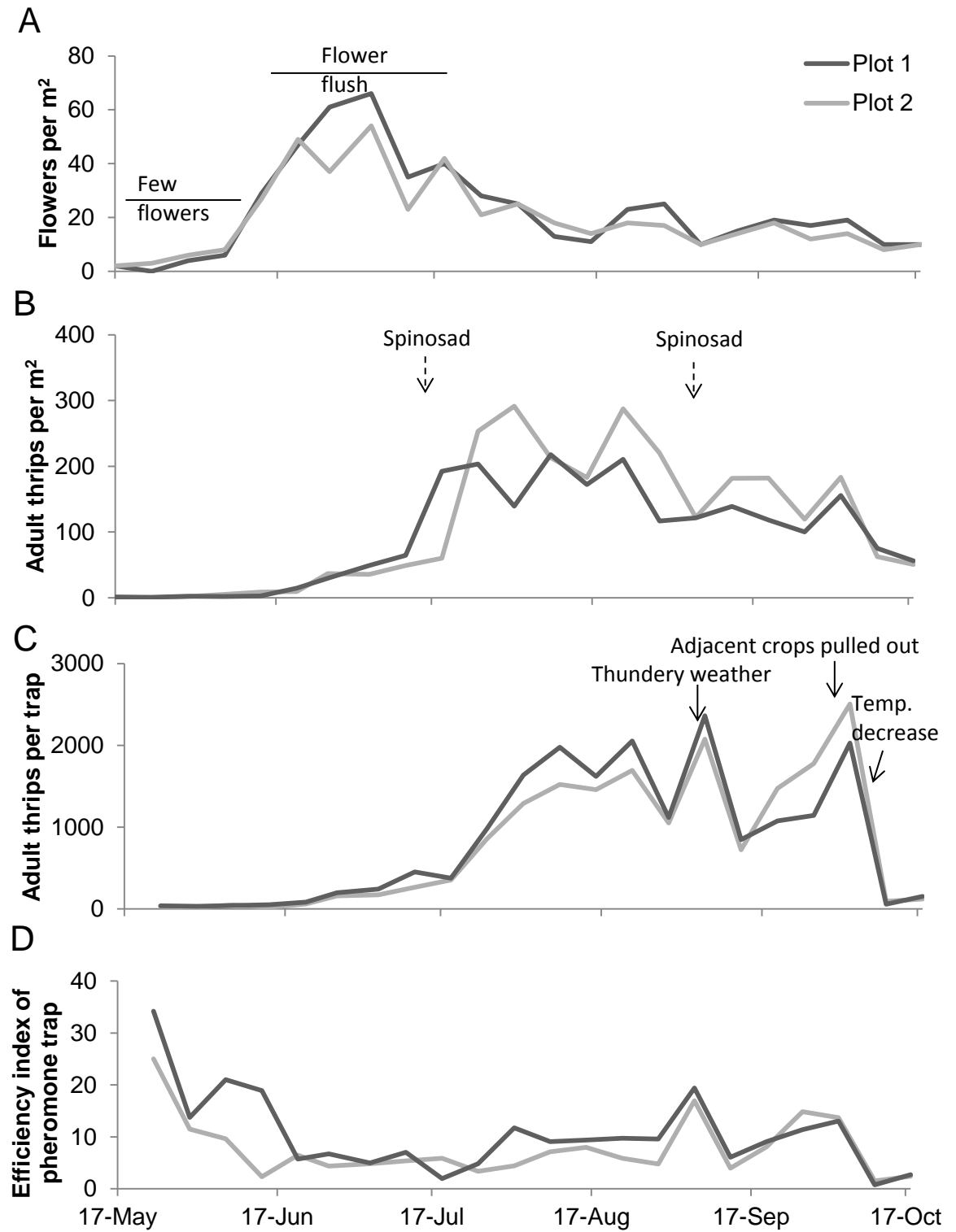


Figure 6.9. Seasonal changes in (A) flowers per m², (B) thrips per m², (C) thrips per trap and (D) trapping efficiency index (thrips per trap/thrips per m²) in two plots in semi-protected strawberry in 2011.

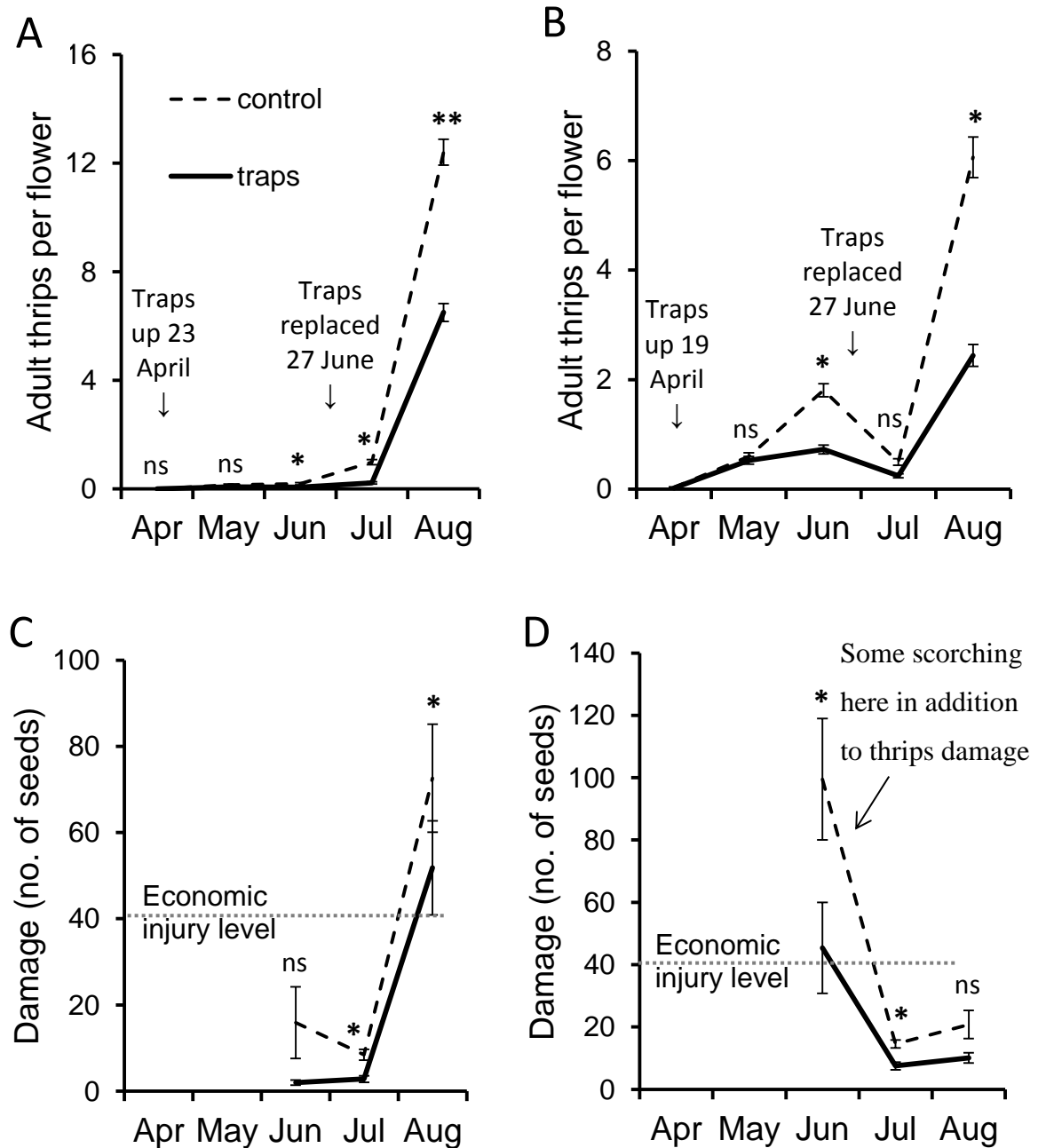


Figure 6.10. Samples taken through the season in semi-protected strawberry in: plots without blue sticky roller traps and plots with blue sticky roller traps, pheromone lures and plant volatile lures. (A) mean adult thrips per flower \pm SEM in a 1st year crop (B) mean adult thrips per flower \pm SEM in a 2nd year crop (C) mean fruit damage \pm SEM in a 1st year crop and (D) mean fruit damage \pm SEM in a 2nd year crop. Damage was recorded as the number of seeds surrounded by bronzing on swollen white fruit. Comparison between control and trapped plots are shown by stars for each assessment date ($n = 4$). Analysis was on transformed data whilst the charts show untransformed data. Key: ns = not significant; * $P < 0.05$; ** $P < 0.01$.

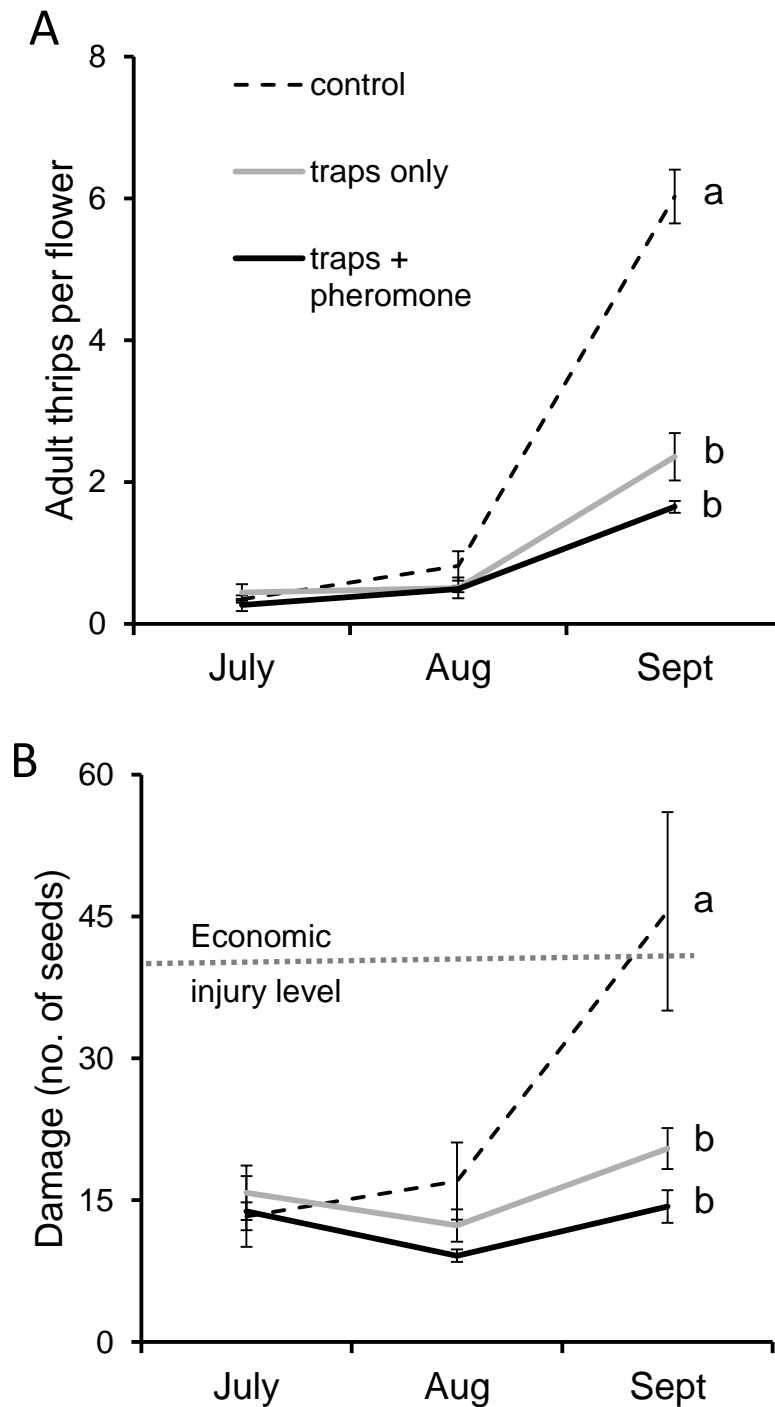


Figure 6.11. Samples taken on 9 July, 8 August and 10 September 2012 in: plots without blue sticky roller traps; plots with blue sticky roller traps only; and plots with blue sticky roller traps and pheromone lures. (A) mean adult thrips per flower \pm SE and (B) mean fruit damage \pm SE. Damage was recorded as the number of seeds surrounded by bronzing on swollen white fruit. Differences were significant in September (thrips numbers $P < 0.001$, fruit damage $P = 0.006$) ($n = 3$). Means with the same letter are not significantly different ($P > 0.05$). Analysis was on transformed data whilst the charts show untransformed data.

Chapter 7

General discussion

The overall aim of this study was to improve the management of *F. occidentalis* in semi-protected strawberry in the UK by developing an easy to use monitoring method with attendant economic injury levels (EILs) and by investigating the viability of using traps for control of *F. occidentalis*. Improvements were made through new knowledge and understanding of the biology of the species. During the study, discoveries were made about the phenology of thrips in UK strawberry, which were investigated further. The use of pheromone for monitoring and mass trapping was investigated.

7.1. Monitoring thrips

The use of action thresholds for decision-making requires an accurate estimate of population density that can be used by growers in the field. Methods of sampling thrips in strawberry flowers and on traps have been developed previously (González-Zamora & Garcia-Marí, 2003; Steiner & Goodwin, 2005b). This study improved the accuracy of assessing thrips populations in flowers by adding to the knowledge of the distribution of thrips on plants. Adult thrips were used as larvae cannot be sampled reliably by eye in the field (González-Zamora & Garcia-Marí, 2003). The age and position of strawberry flowers sampled could affect population estimates of adult thripids by as much as a factor of four (see Chapter 2). This highlighted the importance of taking a consistent flower sample. A reliable estimate of the adult thrips population in strawberry flowers was achieved by farm staff using the revised methods following a minimum of training (Table 2.3). It was sufficient to sample 10 flowers from an area of interest to estimate the thrips density in medium-aged flowers taken from the tops of plants with 80% confidence in the mean. As found by Shipp and Zariffa (1991), monitoring thrips population density in flowers was a better measure than trap catch for predicting fruit damage as it accounted for changes in flower density within a crop and was less subject to weather conditions that affect thrips flight (see Chapter 4).

There have been reports of the estimates of adult thrips per flower using eye counts differing greatly from absolute counts from alcohol samples (J. Fitzgerald, pers. comm., 2013), so the next step is to train growers in the sampling method and to verify it on a wider scale. Within fields, thrips population density correlated with temperature gradients under the polytunnels (Figure 3.8). This information could be used by growers to predict areas of greatest risk of thrips damage and to adapt sampling programmes and control measures accordingly (e.g. numbers of predators released in different areas). Year on year records of pest populations are invaluable for decision-making at a farm level, both to predict periods of risk under local conditions, but also to confirm that low numbers of thrips do not cause economic crop loss when predators are well established. This builds the confidence needed to avoid unnecessary spray treatments. The change from a chemically-based to a biologically-based control programme is a cultural change that relies on knowing the relative numbers of pests and predators in a crop, so the first step towards improved control is to start monitoring as soon as flowering commences (Figure 7.1 B).

7.2. Economic Injury Levels

On strawberry, *F. occidentalis* causes bronzing (russeting) on fruit, which correlates directly with thrips density in flowers (Steiner & Goodwin, 2005a; Coll *et al.*, 2007a; Nondillo *et al.*, 2010). Damage to strawberry fruit has been shown to vary with cultivar, growing system and climate, so it was necessary to quantify fruit bronzing in relation to thrips density in UK crops before EILs could be defined. Data on the susceptibility of different strawberry stages to bronzing is contradictory (most damage occurred either on green fruit, red fruit or on all stages of fruit in the papers above) and the relative damage caused by different thrips stages was untested. This study quantified thrips damage under controlled conditions and in the field, in different months and years (see Chapter 4). All stages of strawberry flower and fruit were susceptible to thrips damage and larvae caused more damage than adults per individual. The timing of damage related most to the numbers and distribution of thrips larvae on different fruit stages at the time. At lower thrips densities, larvae were most abundant at late flowering and early fruit stages, so most damage occurred soon after flowering, but at higher thrips densities there were proportionally more larvae on the later stages of fruit development, which partly explains why the relative timing of damage varies between crops. Shakya *et al.* (2010) found that

the distribution of thrips larvae on strawberry plants varies with predator establishment and pollen availability, but further controlled studies are required to determine whether the change in larval distribution at different thrips densities was due to movement of larvae as a result of competition for food and space, or due to predation or avoidance of predators.

The EILs were derived by regressing fruit damage on thrips density, then calculating the lowest thrips density that would result in economic fruit damage from the regression equations. Economic fruit damage was defined as the amount of bronzing on fruit that would result in the downgrading from a good quality higher-priced (class 1) fruit to a lower-priced (class 2) fruit, which occurred when about 10% of the fruit surface was bronzed (Figure 4.10). The resulting EILs of 5-11 adult thrips per medium-aged flower (see Chapter 4) were similar to those identified in Australia, where temperatures were warmer and EILs might be expected to be lower (Steiner & Goodwin, 2005a). This suggests that although EILs were identified in the West Midlands, they might be more widely applicable to other (warmer) parts of the UK. All thresholds are an approximate guide to be treated with some caution and adjusted at a farm level according to specific biotic and abiotic factors such as temperature, growing methods, secondary pests and their controls at the time. Despite this, the relationship between thrips density and fruit damage was surprisingly consistent, but the studies were based on one cultivar and were carried out in one region of the UK, so further data are required to determine whether the relationship is more widely applicable. Some cultivars favour thrips development (Rahman *et al.*, 2010), but the susceptibility of different cultivars to damage has not been tested, nor have the physical and chemical properties been identified that make some cultivars more favourable than others. This knowledge could identify features that would confer some tolerance or resistance to thrips. The EILs are considered realistic as they are derived from actual damage observed in a commercial pack-house. If anything they are conservative because the damage in the field was assessed on white fruit, where damage shows up more clearly, whereas the damage in the pack-house was assessed on red fruit. The EILs may change when there is a glut or scarcity of good quality fruit, or between pack-houses, or supermarket buyers, but this was not tested.

One of the most effective control strategies currently available to UK strawberry growers for the control of pesticide-resistant *F. occidentalis* is to combine the use of the predatory mite, *N. cucumeris*, which predaes thrips larvae, with the occasional use of spinosad (Tracer) to reduce outbreaks of adult thrips (Rahman *et al.*, 2012). As pesticide-

resistant strains of *F. occidentalis* are widespread (Sparks *et al.*, 2012) and spinosad (Tracer) can interrupt predatory mite establishment (Rahman *et al.*, 2011b), it is important to avoid unnecessary treatments by using action thresholds (Figure 7.1 D, E). Shakya *et al.* (2010), estimated that action thresholds could be relaxed by between 1-2 thrips for each predatory mite present per flower or fruit based on their predation rate. This study goes further by quantifying the reduction in thrips damage to fruit resulting from *N. cucumeris* predation, and the results (relaxation of the threshold by 1.6 thrips per mite) support those of Shakya *et al.* (2010). *Neoseiulus cucumeris* is often considered a rather poor predator because it only feeds on first instar larvae, but this study demonstrates that it prevents fruit damage in strawberry (Figures 4.5, 4.6) by feeding on larvae, thus providing further evidence that larvae are the most damaging thrips stage (Figure 4.7). The study suggests that the EIL can be relaxed from about five adult thrips per flower where few predators are present up to about 11 adult thrips per flower where predatory mites are well established (Table 4.4). Above these thrips densities, adult thrips can cause sufficient damage on their own, without subsequent larvae, which would not be controlled by the predatory mites that only feed on larvae (see Chapter 4). Although the use of *N. cucumeris* is increasing in UK semi-protected strawberry and is routine in glasshouse crops, there are still some research questions that have not been addressed. The sampling methods for predatory mites need to be optimised for strawberry and further studies are required to confirm the percentage cover and numbers of predators required per fruit to prevent damage. Control of *F. occidentalis* using *N. cucumeris* is a preventative treatment that relies on good distribution of the predatory mites over the crop before adult thrips populations build up (Fitzgerald & Jay, 2011). The distribution of mites was patchy in the fields sampled during this study, so improved application methods are required to ensure an even distribution. Methods might include repeated releases, novel release methods or mechanical distribution (Sampson, 1998; Opit *et al.*, 2005).

This study quantified EILs, but growers need to know action thresholds (AT), which is the thrips density at which treatment should be made to prevent damage. For a fast-acting chemical insecticide like spinosad (Tracer), the AT may be close to the EIL. For example, where predatory mite establishment was poor, damage occurred at around five adult thrips per flower, but little damage was observed at four adult thrips per flower in the crops sampled, so an AT of between four and five adult thrips per flower (or 100% flower occupancy) may be appropriate, but this needs to be verified in commercial crops. The

speed of increase of thrips density in flowers is also relevant, which is affected by the time of year, distribution of thrips between flowers and flower density as well as population size. For slower-acting biopesticides, a lower AT may be appropriate, but until an effective biopesticide treatment has been identified in strawberry and the time-lag between treatment and thrips infection determined, an appropriate AT cannot be defined. The adoption of action thresholds for timing insecticide treatments is likely to reduce the number of spray treatments (Figure 7.1 D, E), which would reduce the selection pressure and therefore maintain or improve the efficacy of the few chemical insecticides available to strawberry growers (Denholm & Jespersen, 1998; Contreras *et al.*, 2008), as well as reducing the impact on the natural enemies that are essential for maintaining control (Rahman *et al.*, 2011b). Although it takes a leap of faith to reduce insecticide use for those growers who have come to rely upon them, the economic benefits of doing so when faced with pesticide-resistant thrips have been proved in protected crops across Europe (van Lenteren, 2007; Sampson *et al.*, 2009). These research results provide further evidence to show that natural enemies can be effective.

7.3. Phenology in strawberry

It is easier to maintain control of low thrips populations than to bring high thrips populations under control, so the phenology of *F. occidentalis* was studied in semi-protected strawberry to identify factors affecting thrips population development that could be manipulated by growers to improve control (see Chapter 3). The study showed that *F. occidentalis* is dominant in the crops sampled; displacing the native *Thrips major* as the season progresses. Further data are required to test whether this displacement results from competition between the two species or because *F. occidentalis* are more resistant to chemical insecticides. Population growth of *F. occidentalis* occurred once mean temperatures exceeded about 15°C and declined again once temperatures fell below 15°C at the end of the season, which is consistent with other crops (Gaum *et al.*, 1994). Comparison of temperature records and published data on the development of UK populations of *F. occidentalis* (McDonald *et al.*, 1998) suggests that they can complete about five generations during the growing season under tunnels. The thrips populations reached a plateau during July and August, but maximum thrips density varied between fields. Predator establishment was identified as one limiting factor, but the effect of flower

density on potential thrips population increase and the thrips carrying capacity of a crop were not quantified. The numbers and pattern of flowering could be manipulated as part of a thrips management strategy.

Adult female *F. occidentalis* overwintered in the dying inflorescences of the previous year's crop. The carry-over of thrips from first- to second-year crops resulted in more thrips in second-year crops by a factor of about 40 at the start of the season, but it is not known whether the removal of old inflorescences (and the thrips that they contain) after cropping would reduce this carry-over. Thrips numbers remained low enough in some first-year crops for *N. cucumeris* to maintain control throughout the season, so the viability of growing only one-year crops could be considered as a way of maintaining low thrips numbers, by comparing the cost of re-planting against losses due to thrips in two-year crops. Alternatively, a treatment could be identified that reduces thrips populations at the end of cropping. Slower-acting or longer-residual pesticides (biological or chemical) could be used at this stage as long as they do not interfere with predator establishment the following spring. Although carry-over from first- to second-year crops was demonstrated (Figure 3.7) and thrips are known to be carried between fields on plants and equipment, there is limited information on the extent to which *F. occidentalis* migrates between fields and farms within the UK and this would help growers to design control strategies further.

Frankliniella occidentalis has a wide range of host plants (e.g. Chamberlin *et al.*, 1992). It was found on many weed species within strawberry fields in this study, three of which were common and widespread and flower throughout the year. Weed hosts are another source of thrips infestation and overwintering (Tables 3.4, 3.5). No attempt was made to quantify the effect of weed control on *F. occidentalis* damage, but spring-flowering weeds are likely to increase thrips abundance early in the season. Growers frequently report an invasion of thrips adults and consequent damage following mowing around the edges of fields, as observed in grapevines and other crops (Allsopp, 2010), so mowing or weeding at the wrong time might be a direct cause of damage resulting from adult thrips migrating onto a crop. Where natural enemies are well established, weeds can also be a refuge for predators (Frescata & Mexia, 1996), so the relative benefits and disadvantages of weeds need to be quantified in UK strawberry.

7.4. Optimising the use of the aggregation pheromone

Male *F. occidentalis* produce an aggregation pheromone, neryl (*S*)-2-methylbutanoate, which increases trap-catch (Hamilton *et al.*, 2005), but by relatively small amounts (Table 1.1). The aggregation pheromone was tested at different release rates, chiral forms and in different crops (Chapters 5 and 6), but no improvement in trap catch was identified above those already found (Table 1.1). The experiments added to the knowledge on flight behaviour. The thrips landed in response to pheromone combined with an attractive colour rather than flying directly to a lure. This limits the potential increase in trap-catch as thrips may land on flowers before reaching the traps. Neither pheromone nor an attractive colour drew thrips far above a flowering crop. Thrips may be arrested in flowers that contain food, scent and an attractive colour. Studies suggest that response to pheromone may be greater when there are few flowers (Figure 6.9 D), but further experiments are required to confirm this. Even the doubling of trap catch observed with the aggregation pheromone in strawberry can be used to improve the sensitivity of monitoring traps or to enhance mass trapping (Sampson & Kirk, 2013). The increased activity resulting from the aggregation pheromone (Olaniran, 2013) has the potential to improve the efficacy of insecticides by increasing the pick-up of a chemical, or increasing exposure to the sprays in a similar way to alarm pheromone (Cook *et al.*, 2002), or it could increase the efficacy of attract and kill or attract and infect techniques (Niassy *et al.*, 2012).

A second male-produced volatile, (*R*)-lavandulyl acetate, has been shown to calm females and may have a role in mating behaviour (Olaniran, 2013), or it may be part of the aggregation pheromone at a specific concentration or ratio to neryl (*S*)-2-methylbutanoate (Zhu *et al.*, 2012). Different release rates, ratios and chiral forms of lavandulyl acetate were tested in the field and (*R*)-lavandulyl acetate consistently reduced the trap catch of female *F. occidentalis*, supporting its role as a calming pheromone, but there was no evidence that it is part of the aggregation pheromone. Further experiments are required to elucidate its role and to test whether the arrestment effect is strong enough to reduce feeding or egg-laying, which might have an applied use in crop protection. Male-produced aggregation and sex pheromones can involve a complex of compounds that have different roles at long and short range (Sirugue *et al.*, 1992). *Frankliniella occidentalis* adult males produce several less-volatile hydrocarbons, including 7-methyl tricosane that causes females to stay in the vicinity of the pheromone on contact (Olaniran *et al.*, 2013). Thrips

escape from sticky traps (Figure 6.5), so it is possible that such pheromones could reduce escape-rate.

7.5. Mass trapping

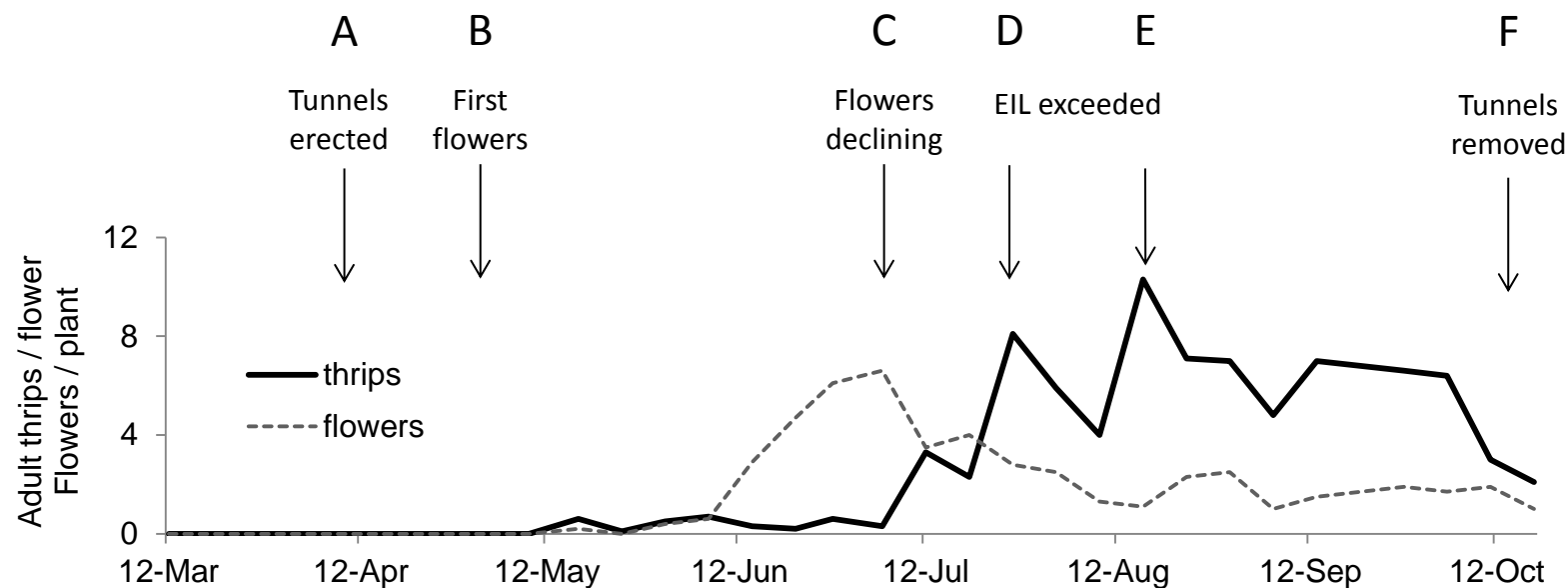
A practical method of thrips mass trapping was developed for semi-protected strawberry that caught sufficient thrips to reduce strawberry fruit damage and increase grower returns when used as part of an IPM programme (Sampson & Kirk, 2013). It took several weeks for the traps to catch sufficient thrips to reduce a population, so it is likely that they can only be used as a preventative measure (Figure 7.1 A, C). The traps are not sufficient to control thrips on their own but are an extra tool for use in addition to the biological (e.g. predatory mites) and chemical (e.g. spinosad) control methods that are already used and will strengthen IPM programmes. Trapping complements *N. cucumeris* by controlling adults, whereas *N. cucumeris* only feeds on larvae. It also reduces the need for remedial treatments, so makes a useful contribution to the management of insecticide resistance.

There is scope for improving mass trapping by optimising trap colours, glues, scents distance between lures and replacement rate, although the impact on important beneficial insects would have to be tested to ensure compatibility. The traps reduced thrips numbers and fruit damage from June to September, when daytime temperatures were optimal for thrips flight. Further research is needed to test whether traps could be useful throughout the year. Although temperatures are usually below the flight threshold of about 15°C through the winter, flight still occurred on the occasional warm day (see Chapter 3) and the relative attraction of traps may be greater in the winter when there are fewer flowers to compete with the traps. Trapping efficiency may be affected by cultural techniques. Some growers in Southern UK use blue mulches, which could attract thrips away from blue sticky traps and reduce trap-catch. In this study, roller traps were placed down every tunnel (6.5-8.0 m apart) and further information is required on the flight behaviour of thrips and the distance of attraction to determine whether the traps could be spaced wider apart without reducing the efficacy. If significant numbers of thrips migrate into first-year crops from nearby fields, then surrounding the crops with traps may be effective. If mass trapping is adopted more widely by growers, then an automated, tractor-mounted application system could be developed to reduce the application costs.

7.6. Integrated Pest Management (IPM) of *F. occidentalis* in semi-protected strawberry

During the course of this project a grower who has implemented some of the strategies in this study (Figure 7.1), including monitoring, regular predator release and mass trapping, has significantly improved thrips control in semi-protected strawberry, while other growers are still suffering devastating crop loss. Some of the control failures may be due to the difficult transition from chemically-based to biologically-based control methods, as many UK strawberry growers are new to the use of *N. cucumeris*. Evidence for this is the numbers of UK strawberry hectares treated with *N. cucumeris*, which increased from 469 ha in 2006 to 2,567 ha in 2012 (Garthwaite *et al.*, 2006, 2013). Some control failures result from releasing too few predators too late, but at some farms predators are being killed by the use of incompatible pesticide treatments used against thrips and against other pests such as spider mites (*T. urticae*), capsids (*L. rugulipennis*) and spotted wing drosophila (*Drosophila suzukii*). A variety of IPM compatible control methods are available to control these species (Saville *et al.*, 2013) and it is essential to avoid the use of broad-spectrum pesticides during the growing season if pesticide-resistant thrips are to be controlled. Even when the best management programmes are implemented, thrips control occasionally breaks down and growers need fast-acting remedial treatments for such occasions until IPM programmes become more robust. Further research is required to identify remedial treatments that could be rotated with spinosad. These could include new chemistry, biopesticides, or if production costs came down, *Orius* sp. could be released at inundative rather than inoculative rates. The UK has been at the forefront of developing biological pest control techniques in greenhouse crops that result in sustainable pest control to the benefit of growers, crop workers and consumers (van Lenteren, 2007). Whereas the use of natural enemies has become routine in most glasshouse crops, the technology still needs to be transferred to those growers and also to some advisers who are new to biological control, so training and technology transfer may be as important as research to improve *F. occidentalis* control in UK semi-protected strawberry crops.

Figure 7.1. How the methods identified in this study might be used to improve the control of *F. occidentalis* in semi-protected strawberry.



Possible actions:

A: Add roller traps for mass trapping to the polytunnel 'legs' as soon as the cladding goes on as warmer temperatures will increase thrips flight.

B: Start monitoring weekly and record the numbers of adult thrips per flower, the numbers of flowers per plant and predator establishment.

Establish the predatory mite *Neoseiulus cucumeris* from first flowering and repeat releases as required.

C: A period of risk when flower numbers are declining, monitor carefully and replace the roller traps.

D and E: If the numbers of adult thrips per flower reach the EIL (economic injury level), treat with a compatible pesticide.

F: At the end of cropping, the impact of removing senescent flower trusses and controlling weeds on spring thrips numbers could be tested.

8. References

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Appendix A: Handout given to farm staff before sampling thrips in flowers (see Chapter 2, 2.5.1.2).

1. Select one **medium** aged flower sticking up from the **top** (not side) of each plant. Medium aged flowers have fresh looking petals but the pollen has dropped from the anthers, so the anthers look a bit darker.

Young flower
(yellow anthers)



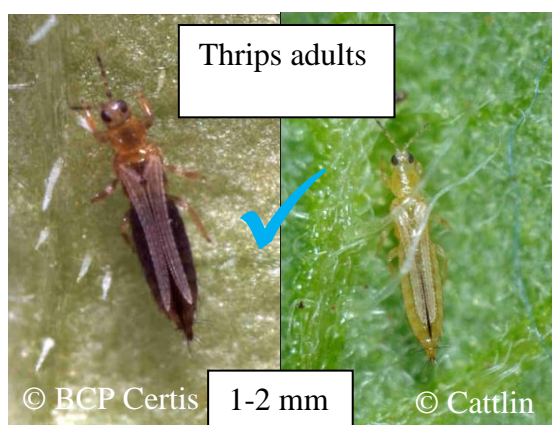
Medium aged flower
(brown anthers)



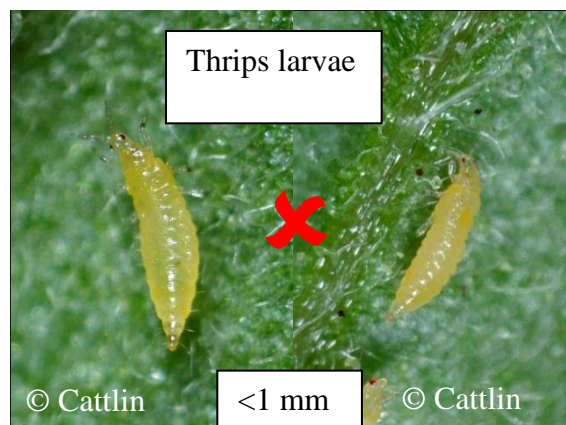
Senescent flower
(dropped petals)



2. Count the numbers of **adult** thrips (do not count larvae) in each flower and record them on the sheet. Use a x 10 hand lens to see the thrips. Carefully pull down the petals on each side of the flower to see the thrips.

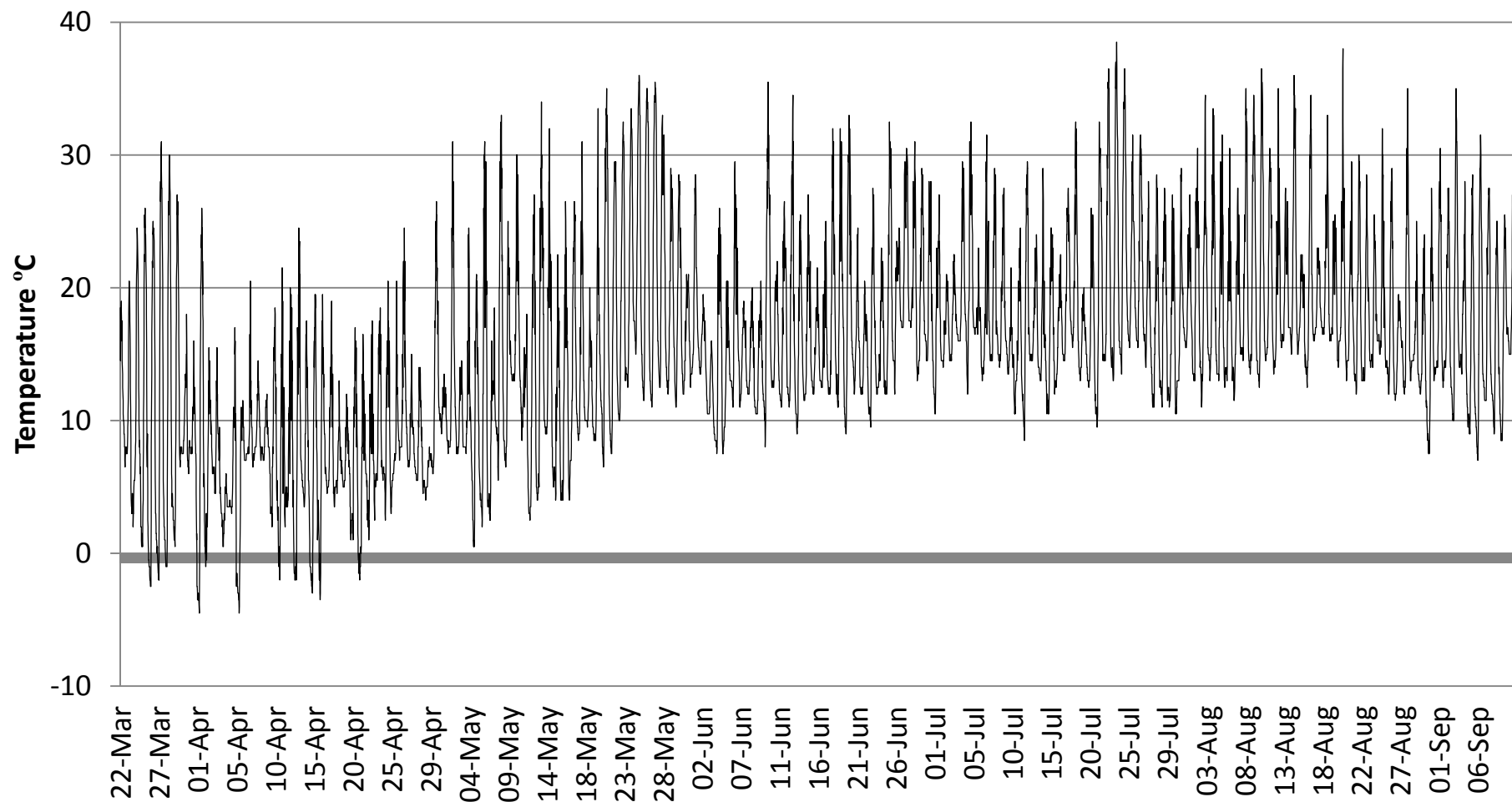


Adult thrips have wings, may be dark or pale



Thrips larvae have no wings, all yellow

Thrips photographs kindly provided by BCP Certis and Nigel Cattlin, Holt Studios



Appendix B: Temperatures recorded with a data logger placed inside a delta trap at about flower height during the growing season, in a semi-protected strawberry crop (field 3 in table 2.1) in the West Midlands, 2012.